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# Biology of Dwarf Mistletoes:

## Proceedings of the Symposium



August 8, 1984  
Fort Collins, Colorado

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## Preface

"So much has already been written on this genus of the Loranthaceae that many readers...will no doubt be surprised that there should be anything new to be said on the subject." This profoundly non-prophetic statement was written by T. Johnson in an article on Arceuthobium oxycedri in the Annals of Botany in 1888. Indeed, much more has been written on the subject in the ensuing century (at least 3,500 references) but much more remains to be learned about this fascinating genus, the most advanced of the mistletoes.

The dwarf mistletoes are the most damaging group of parasitic seed plants in North America. They parasitize several species of conifers, including the most important commercial timber trees. The group is also very interesting from a biological standpoint because of the long period of co-evolution of host and parasite. The dwarf mistletoes are extremely reduced morphologically, very host specific, and are nearly holo-parasitic. The dwarf mistletoes were the subject of a USDA Forest Service-sponsored symposium in 1978, but that symposium focused on control through forest management, not basic biology. The National

Institute of Biological Sciences meeting at Colorado State University, Fort Collins, provided an opportunity for biologists from various disciplines to meet and discuss the biology of the dwarf mistletoes.

Rapid publication of these proceedings was due largely to the excellent efforts of the authors (and their typists!) in preparing camera-ready manuscripts. Since papers are being printed as received, each contributor is responsible for the accuracy of his or her paper; opinions expressed by the authors may not necessarily reflect the policy of the U.S. Department of Agriculture.

Many people contributed to the success of the symposium. The original suggestion to hold the symposium was made by Clyde L. Calvin, of Portland State University. We thank the many scientists who kindly provided reviews of manuscripts for these proceedings: Neil A. Anderson, Clyde L. Calvin, M. Thomas Conkle, William B. Critchfield, Leanne Egeland, Carl Eide, George T. Ferrell, F. Thomas Ledig, Arthur H. McCain, Michael E. Ostry, and Richard S. Smith. R. H. Hamre compiled the proceedings for publications.

Cover: A recently described dwarf mistletoe, Arceuthobium pendens, a rare parasite of pinyons in Mexico (see p. 5). Drawing by Christine Dixon.



# BIOLOGY OF DWARF MISTLETOES: PROCEEDINGS OF THE SYMPOSIUM

August 8, 1984  
Colorado State University,  
Fort Collins

Frank G. Hawksworth and Robert F. Scharpf  
Technical Coordinators

Sponsored by:

Rocky Mountain and Pacific Southwest Forest and Range Experiment Stations  
USDA Forest Service

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# Dwarf Mistletoes: Insidious Pests of North American Conifers

Ed F. Wicker<sup>1</sup>

Dwarf mistletoes are parasitic seed plants of the genus Arceuthobium. Members of this genus have not attained the symbolic or historical significance of other taxa of the family Viscaceae but they excel as destructive pathogens of forest trees in the family Pinaceae. Several species are native to North America. Although genesis of the taxa likely surpasses that of their hosts in antiquity, the two have co-evolved over several million years. The genus reaches its greatest degree of diversity in western North America.

Recorded observations on the parasitic behavior of mistletoes date to Theophrastus, 305 B.C. (Gill 1953). The earliest written record of dwarf mistletoe discovered so far was that of Clusius in 1601 from southern Europe (Hawksworth 1978). The parasite was not reported from North America until the early 1800's. For the next 100 years or so, interest on these plants was driven mainly by botanical curiosity.

Although the importance of dwarf mistletoes as destructive pathogens in western coniferous forests was documented in the early part of the twentieth century (Weir 1916a, b), they remain a most important forest disease problem. Our best estimate for annual losses of timber products attributable to dwarf mistletoes in the western United States now stands at 3.3 billion board feet. On a regional basis, it has been estimated that 50 percent of the lodgepole pine and Douglas-fir forest and 35 percent of the ponderosa pine forest of the Central and Southern Rockies are affected by dwarf mistletoes. Some stands are so severely damaged that they are producing at less than 1/3 potential. Such production certainly cannot be considered economically feasible management. Estimates of annual losses of timber products in the Northern Rockies attributed to dwarf mistletoes exceed those for the Central and

Southern Rockies. Possible losses in tree seed production, product quality, predisposition to other pests, recreation and aesthetic values, and wildlife habitat have not been reported.

Silvicultural practices can be used to reduce intensity of dwarf mistletoe to non-pest levels. Such practices are being implemented on millions of acres of commercial forest lands in the western United States, and good progress is being made in reducing productivity losses to dwarf mistletoes. This control program is in constant need of refinement to stay in step with changing needs and demands of society. Increased knowledge of the biology and ecology of the pest will prove invaluable in meeting these demands for change in the control program.

I would like to close with a word of caution. We have a long way to go in this war with dwarf mistletoe. Silvicultural controls take time, and that time can work both for and against us. Dwarf mistletoe is a slow, insidious pest that fights a war of attrition. It wears down our interest, the visibility of our efforts, and thus the financial support for our control programs. The best insurance to avoid this happening is for research to (1) rapidly develop the capability to accurately quantify production losses caused by dwarf mistletoe, and (2) respond quickly to changing economic and social demands that may require changes in on-going control efforts.

These needs are long-standing, and so far research has been slow to respond. Consequently, we are paying the price in terms of dwindling support. It is imperative that we look on this symposium on biology of dwarf mistletoes as a building block in our fight to achieve effective, long-term control of an exceedingly costly, tenacious forest pest!

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# Biology and Classification of *Arceuthobium*: an update<sup>1</sup>

Frank G. Hawksworth and Delbert Wiens<sup>2</sup>

**Abstract.**--*Arceuthobium* is the only mistletoe genus that occurs in both the New and Old Worlds. In our 1972 monograph, we recognized 32 taxa; 28 in the New World and 4 in the Old. Since then, 9 new taxa have been described or recognized; 5 in the New World and 4 in the Old. Most of the new taxa in the New World are on *Pinus*: *A. aureum* ssp. *aureum* in Guatemala and Belize; *A. aureum* ssp. *petersonii* in southern Mexico; *A. globosum* ssp. *grandicaule* in Mexico and Guatemala, and *A. pendens* in central Mexico. The most surprising is a new species, *A. cubense*, on *Podocarpus* in eastern Cuba, the first report of a host of *Arceuthobium* in the Podocarpaceae. This is so exceptional that verification is needed. In the Old World, the recently described or recognized taxa are: *A. azoricum* on *Juniperus* in the Azores; *A. juniperi-proceræ* on *Juniperus* in Ethiopia and Kenya; *A. tibetense* on *Abies* in southwestern China, and *A. pini* var. *sichuanense* on *Picea* in southwestern China. The greatest increase in the number of known taxa since 1972 has been in China (from 2 to 6), Guatemala (2 to 5), and Mexico (16 to 19). *Arceuthobium* is reported for the first time in El Salvador and Cuba. *Pinus* is the primary host of the genus (25 of 41 taxa), but for only 2 of the 8 Old World taxa.

## Introduction

*Arceuthobium* is the only mistletoe genus that occurs in both the eastern and western hemispheres. The first report of dwarf mistletoe was by Clusius (1576), who described "*Viscum oxycedri*" on junipers in the Mediterranean area. The first *Arceuthobium* in the New World was described by Willdenow, as "*Viscum vaginatum*," from central Mexico in 1806. *Arceuthobium* was established as a separate genus by Marschall von Bieberstein in 1819. Details on the taxonomic history of the genus are given by Hawksworth and Wiens (1972). Much of the early literature on the genus was taxonomic, although some pioneer forest pathologists, such as Drs. J. R. Weir and L. S. Gill recognized the significant pathological effects caused by these parasites.

The dwarf mistletoes, because of their unique biology and great economic importance, are receiving increasing attention in the literature. In the latest edition of our FAMULUS information retrieval system of world mistletoe literature (Scharpf et al. 1976), we have nearly 3500 titles on *Arceuthobium*. During the first half of the 20th century, there were only 10 publications per year on the group.

<sup>1</sup>Paper presented at the Symposium on Biology of Dwarf Mistletoes, in conjunction with the A.I.B.S. Meetings, August 8, 1984, Fort Collins, Colorado.

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Since 1950, however, the increase has been dramatic:

Dates	Number of <i>Arceuthobium</i> publications/year
1950-1954	26
1955-1959	37
1960-1964	57
1965-1969	81
1970-1974	112
1975-1979	119

The number of publications appearing in the early 1980's indicates that this increasing trend is continuing.

Little taxonomic research on the genus has been undertaken since our 1972 monograph. Crawford and Hawksworth (1979) examined the flavanoid compounds from 36 taxa. The flavanoid chemistry of the genus is rather uniform, all taxa producing 3-O-glucosides of the flavonols quercetin and myricetin. No infraspecific chemical variation was encountered, and, in those instances where subspecific taxa are recognized, their chemistry was uniform. At the subgeneric level, members of subgenus *Arceuthobium* synthesize primarily glucosides, whereas galactosides are more common in subgenus *Vaginata*. In two of the four Old World species of subgenus *Arceuthobium* (*A. juniperi-proceræ* and *A. oxycedri*) only myricetin 3-O-glucoside was detected. There are no absolute flavanoid differences between subgenera, sections, or series. Flavonoids, however, are useful in several instances at the species level. In several cases, chemical data lend support to the

Table 1.--Synopsis of the genus *Arceuthobium*.

I. Subgenus *Vaginata* Hawksw. & Wiens

A. Section *Vaginata*

1. *A. aureum* Hawksw. & Wiens ssp. *aureum*
2. *A. aureum* ssp. *petersonii* Hawksw. & Wiens
3. *A. gillii* Hawksw. & Wiens ssp. *gillii*
4. *A. gillii* ssp. *nigrum* Hawksw. & Wiens
5. *A. globosum* Hawksw. & Wiens ssp. *globosum*
6. *A. globosum* ssp. *grandicaule* Hawksw. & Wiens
7. *A. vaginatum* (Willd.) Presl ssp. *vaginatum*
8. *A. vaginatum* ssp. *cryptopodum* (Engelm.) Hawks. & Wiens
9. *A. vaginatum* ssp. *durangense* Hawksw. & Wiens

B. Section *Campylopoda* Hawksw. & Wiens

a. Series *Campylopoda*

10. *A. abietinum* (Engelm.) Hawksw. & Wiens f. sp. *concoloris* Hawksw. & Wiens
11. *A. abietinum* (Engelm.) Hawksw. & Wiens f. sp. *magnificae* Hawksw. & Wiens
12. *A. apachecum* Hawksw. & Wiens
13. *A. blumeri* A. Nels.
14. *A. californicum* Hawksw. & Wiens
15. *A. campylopodum* Engelm.
16. *A. cyanocarpum* Coulter & A. Nels.
17. *A. divaricatum* Engelm.
18. *A. guatemalense* Hawksw. & Wiens
19. *A. laricis* (Piper) St. John
20. *A. microcarpum* (Engelm.) Hawksw. & Wiens
21. *A. occidentale* Engelm.
22. *A. pendens* Hawksw. & Wiens
23. *A. tsugense* (Rosendahl) G. N. Jones  
Hemlock race  
Shore pine race

b. Series *Rubra* Hawksw. & Wiens

24. *A. bicarinatum* Urban
25. *A. hondurensense* Hawksw. & Wiens
26. *A. rubrum* Hawksw. & Wiens

c. Series *Stricta* Hawksw. & Wiens

27. *A. strictum* Hawksw. & Wiens

C. Section *Minuta* Hawksw. & Wiens

28. *A. douglasii* Engelm.
29. *A. pusillum* Peck

II. Subgenus *Arceuthobium* (No sections designated.)

New World Species

30. *A. abietis-religiosae* Heil
31. *A. americanum* Nutt. ex Engelm.
32. *A. verticilliflorum* Engelm.

Old World Species

33. *A. azoricum* Wiens & Hawksw.
34. *A. chinense* Lecomte
35. *A. minutissimum* Hook.
36. *A. juniperi-procerae* Chiov.
37. *A. oxycedri* (DC.) M. Bieb.
38. *A. pini* Hawksw. & Wiens var. *pini*
39. *A. pini* var. *sichuanense* H. S. Kiu
40. *A. tibetense* H. S. Kiu & W. Ren

III. Status unknown

41. *A. cubense* Leiva & Bisce

recognition of species that in the past have been considered doubtfully distinct on the basis of morphology: *A. apachecum*, *A. blumeri*, *A. cyanocarpum*, *A. californicum*, and *A. microcarpum*. But, the flavonol chemistry is identical in other closely related species such as *A. campylopodum* and *A. occidentale*.

*Arceuthobium* has been separated into two subgenera based primarily on branching patterns: verticillate in subgenus *Arceuthobium* and flabellate in subgenus *Vaginatum* (Hawksworth and Wiens 1972). Mark and Hawksworth (1981) studied two California members of subgenus *Vaginatum* (*A. occidentale* and *A. campylopodum*) and showed that they exhibited limited (average less than 5%) verticillate branching, in addition to the pre-dominant flabellate type. Presence of flabellate branching, rather than absence of verticillate branching, is thus a better criterion for distinguishing subgenus *Vaginatum*. Subgenus *Arceuthobium* is apparently exclusively verticillate.

Hawksworth (1982) has published a taxonomic summary of the genus in Mexico and Central America, along with lists of hosts and distribution maps. Barlow (1983) summarized the biogeography of the genus.

In our 1972 monograph (Hawksworth and Wiens 1972), we recognized 32 taxa in *Arceuthobium*; 28 in the New World and 4 in the Old. Since then, 9 new taxa have been recognized or described: 5 in the New World and 4 in the Old.

NEW TAXA SINCE 1972

New World

In our 1972 paper, we suggested that "*Arceuthobium globosum*" might be divisible into other taxa. Subsequent field studies in Mexico and Guatemala show that this is indeed the case, and we now recognize this complex as consisting of at least 2 species, with 2 subspecies each: *A. globosum* (subsp. *globosum* and *grandicaule*), and *A. aureum* (subsp. *aureum* and *petersonii*) (Hawksworth and Wiens 1977).

1. *ARCEUTHOBIUM GLOBOSUM* subsp. *GRANDICAULE*.<sup>3</sup> This subspecies is widespread in central Mexico and in the highlands of western Guatemala on at least 10 species of pines (fig. 1). It is the largest *Arceuthobium*, with shoots up to 70 cm tall and 5 cm in diameter at the base. It frequently sprouts from the trunks of pines.

2. *ARCEUTHOBIUM AUREUM* subsp. *AUREUM*. This species occurs on pines in low elevations (900-2000 m.) in Guatemala and Belize (fig. 2). It is apparently unique among the New World dwarf mistletoes in that it flowers and fruits continuously throughout the year. Like *A. globosum* subsp. *globosum* in northern Mexico, this taxon does not induce witches' broom formation.

<sup>3</sup>Authorities are given in Table 1.



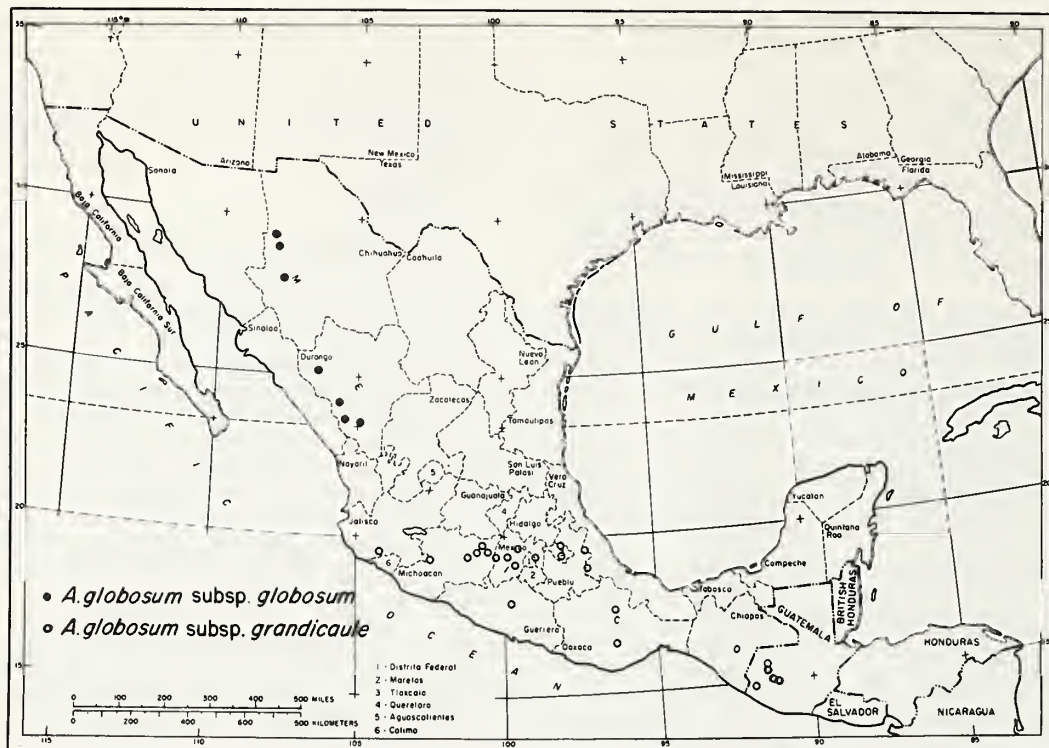


Figure 1.--Distribution of the two subspecies of Arceuthobium globosum in Mexico and Guatemala.



Figure 2.--Distribution of the two subspecies of Arceuthobium aureum in Mexico, Guatemala, and Belize.

3. ARCEUTHOBIUM AUREUM subsp. PETERSONII. This local taxon is known only in Chiapas, Mexico, where it parasitizes at least 6 species of pines (fig. 1). Plants of this taxon are nearly twice the size of subsp. aureum and it sometimes forms witches' brooms.

4. ARCEUTHOBIUM PENDENS. This species parasitizes pinyons and is presently known from only two locations in Mexico: San Luis Potosí on Pinus discolor, and in Veracruz on P. cembroides subsp. orizabensis (Hawksworth and Wiens 1980). It is distinguished by its tall, slender shoots (up to 22 cm) and formation of systemic witches' brooms by the staminate (but apparently not the pistillate) plants.

5. ARCEUTHOBIUM CUBENSE. The recent report of this species, a parasite of the endemic Podocarpus ekmanii Urban in eastern Cuba, is a surprise (Leiva and Bisse 1983). This is the first report of Arceuthobium on hosts outside the Pinaceae or Cupressaceae.<sup>4</sup> The species was formerly classed as Dendrophthora cupressoides, a widespread Caribbean species on several hosts including Juniperus (Kuijt 1961). We have seen no specimens of A. cubense so we are unable to comment on its validity; however, the report is so exceptional that verification is needed.

#### Old World

In our 1972 monograph we mentioned that the vast geographic range and widely disjunct populations in A. oxycedri might necessitate recognition of additional species. This is the case, and we now recognize 3 species formerly included under "A. oxycedri": A. oxycedri sensu stricto on several species of junipers from the Mediterranean area through the Middle East to China; A. juniperi-procerae on Juniperus procera in Kenya and Ethiopia, and A. azoricum on J. brevifolia in the Azores (Hawksworth and Wiens 1976).

6. ARCEUTHOBIUM AZORICUM. This species is restricted to the endemic Juniperus brevifolia on several islands of the Azores (Hawksworth and Wiens 1976). It is unique because of its distinct habit, especially its thick shoots--nearly 1 cm in diameter, which is 2 to 3 times larger than A. oxycedri or A. juniper-procerae, and the very high proportion of 4-merous flowers.

7. ARCEUTHOBIUM JUNIPERI-PROCERAE. This species, a parasite on Juniperus procera, was described by Chiovenda (1911). Our studies in Ethiopia and Kenya show that it differs from A. oxycedri sensu stricto in several respects: occurrence of whorled branching, phenology, shoot color, and shoot glaucousness (Hawksworth and Wiens 1976). This species is the only member of the genus that occurs south of the equator. Possibly this species occurs in Juniperus procera north of the Red Sea in Yemen.

<sup>4</sup>Arceuthobium dacrydii, described on Podocarpus from Java, has since been shown to be a Korthalsella (Hawksworth and Wiens 1972).

8. ARCEUTHOBIUM TIBETENSE. This species was recently described from Xizang (Tibet) where it is a local parasite of Abies georgei (Kiu and Ren 1982, Kiu 1984).

9. ARCEUTHOBIUM PINI var. SICHUANENSE. This parasite of Picea has a broad distribution in southwestern China (Sichuan and Tibet) (Kiu 1984). A distribution map for it is given by Kiu and Ren (1982). Variety sichuanense is much smaller than A. pini var. pini on Pinus.

#### STATUS OF PREVIOUSLY KNOWN TAXA

Here we summarize the current taxonomic status and significant extensions in geographic or host ranges since publication of our 1972 monograph (Hawksworth and Wiens 1972).

#### New World

1. ARCEUTHOBIUM ABIETINUM f. sp. CONCOLORIS. This mistletoe parasitizes Abies concolor and A. grandis, primarily in California and Oregon, with isolated locations in Washington, Nevada, Utah, and Arizona. No new hosts have been reported for this mistletoe. Several new localities for it have been reported (fig. 3), especially in Oregon, Utah, Nevada, and in southern Arizona (Mathiasen 1976, Mathiasen and Jones 1983). The latter are of particular interest as they represent range extensions of about 500 km.

2. ARCEUTHOBIUM ABIETINUM f. sp. MAGNIFICA. This taxon parasitizes Abies magnifica in California and this host and A. procerae in southern Oregon. A few new localities have been reported but all are within the limits of the range of the taxon as known in 1972.

3. ARCEUTHOBIUM ABIETIS-RELIGIOSAE. This parasite of Abies is common in central Mexico. Since 1972, the mistletoe has been reported in four additional states: Michoacán, Puebla, Tamaulipas, and Tlaxcala (Hawksworth and Wiens 1977).

4. ARCEUTHOBIUM AMERICANUM. This is one of the most widely distributed dwarf mistletoes in North America, ranging from California and Colorado to British Columbia on Pinus contorta, and eastward on P. banksiana to Ontario. Pseudotsuga menziesii is a rare host in Alberta (Muir 1973); it was previously reported as rare on this tree in Utah (Hawksworth and Wiens 1972). The parasite has been found on P. contorta ssp. contorta in a few locations in coastal British Columbia (Smith and Wass 1979), but we have not been able to confirm the early report of A. americanum on this subspecies in the Oregon Mountain area of southwestern Oregon. Several new locations for A. americanum have been reported (fig. 4). The mistletoe is now known from most of the isolated populations of Pinus contorta in central Montana (all except the Highwood and Big Snowy mountains--Dooling and Eder 1981). A detailed distribution map for A. americanum in British Columbia has been published by Baranyay and Bauman (1972). No new locations have been found between the eastern limits of the mistletoe in southeastern Manitoba and the disjunct locality near Lac Seul in Ontario.



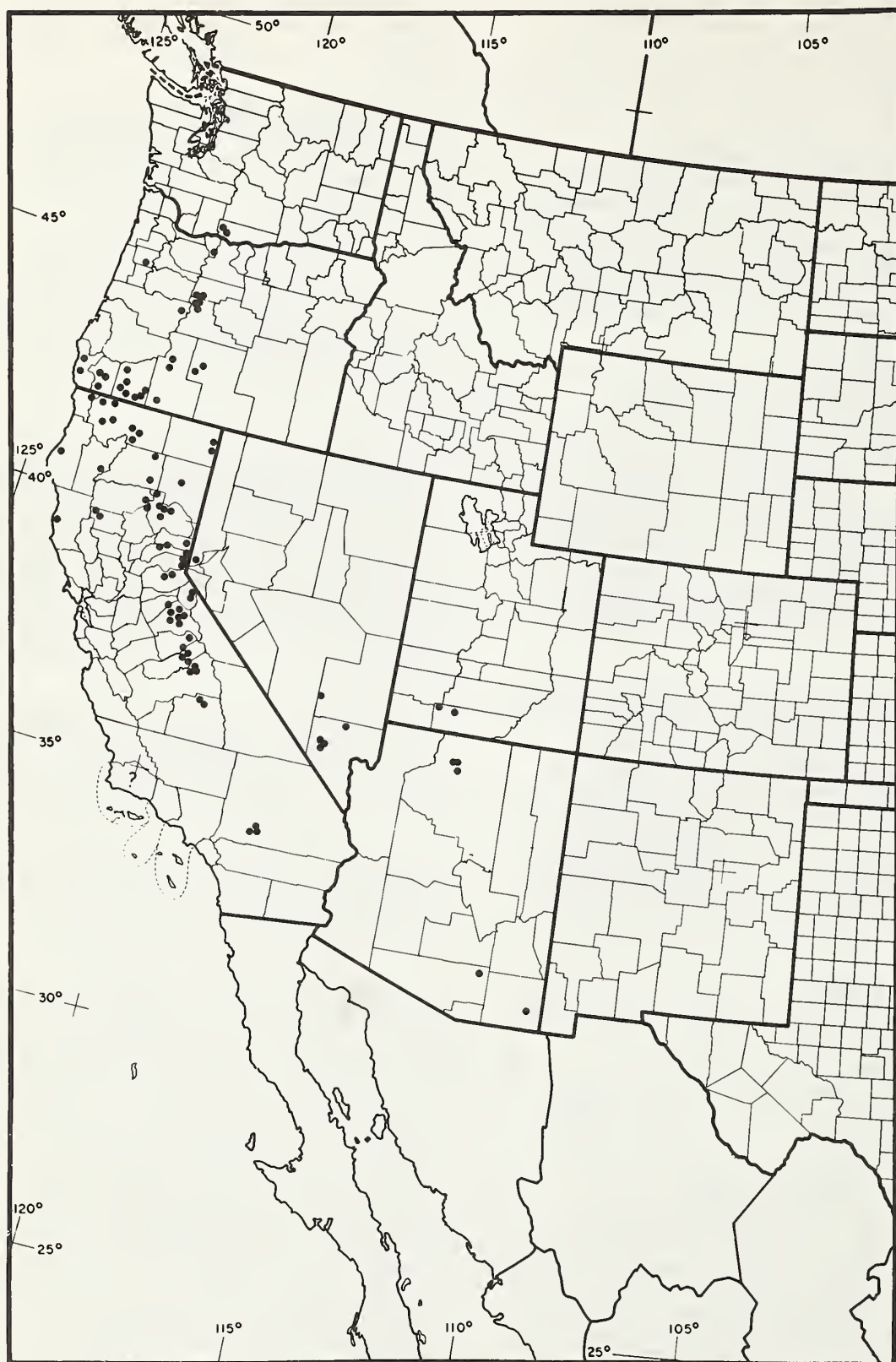


Figure 3.--Distribution of *Arceuthobium abietinum* f. sp. *concoloris*. Smith's (1976) reports on Big Pine Mountain and Reyes Peak near Santa Barbara have not been confirmed.



Figure 4.--Distribution of *Arceuthobium americanum* in the western United States.

Due to fire and logging, this local population in Ontario may be extinct (Myren and Gross 1977).

5. ARCEUTHOBIUM APACHECUM. No major extensions in the range of this local parasite of Pinus strobiformis in Arizona, New Mexico, and Coahuila, Mexico have been found (Mathiasen 1979). However, in inoculation tests, Pinus flexilis was found to be susceptible to A. apachecum (Mathiasen 1978). The populations that we classified as A. blumeri in the Santa Rita Mountains in southern Arizona have since been found to be A. apachecum (Mathiasen 1982).

6. ARCEUTHOBIUM BICARINATUM. No new information on this parasite of Pinus occidentalis in Hispaniola has been reported.

7. ARCEUTHOBIUM BLUMERI. This is a local parasite of Pinus strobiformis in northern Mexico and in a single locality in the United States (Huachuca Mountains, Arizona). Mathiasen (1979), (1982) reports an additional native host: P. ayacahuite var. brachyptera in Durango, Mexico, and also found, by greenhouse inoculations, that P. flexilis is susceptible (Mathiasen 1978). The parasite is also reported for the first time in Sonora (Ajo Mountains--Mathiasen 1979). The species has been found in a few new locations in Mexico, but mostly near previously known localities (Cibrian T. et al. 1980, Hawksworth and Cibrian T. 1984).

8. ARCEUTHOBIUM CALIFORNICUM. This parasite is common on Pinus lambertiana and P. monticola in California and Oregon. The only major range extension is a report on Big Pine Mountain near Santa Barbara (Smith 1976), but no specimens from that area have been seen.

9. ARCEUTHOBIUM CAMPYLOPODUM. This widespread parasite, mainly of Pinus ponderosa and P. jeffreyi, is found from northern Washington and western Idaho to Baja California, Mexico. The only new host reported is the introduced Pinus halepensis, in the Angeles National Forest, California (collected by R. F. Scharpf in 1972, specimen at FPF). A few new locations have been reported, but most are within the geographic range known in 1972. An exception is a collection in the Wallamette Valley of Oregon, about 10 miles south of Corvallis. A collection was made in 1942 by L. F. Roth, and we recently found the mistletoe in the same area. However, ponderosa pine has been severely logged in this area and the mistletoe population is surviving only on a few old-growth trees. This population will probably soon become extinct.

10. ARCEUTHOBIUM CYANOCARPUM. This is a widespread, but local, parasite primarily of Pinus flexilis (and to a lesser extent of P. aristata) in the western United States (fig. 5). Two locations previously thought to be A. cyanocarpum are now known to be other taxa, (1) the San Francisco Peaks population in Arizona is A. microcarpum (Mathiasen and Hawksworth 1980), and (2) the Hawley Lake, Sierra County, California collection is A. tsugense. Several new

locations of A. cyanocarpum have been found (fig. 3) including the first verified report in Oregon (Three Creek Lakes, Deschutes County--Knutson and Tinnin 1981); the Panamint Mountains, California (Johnson 1976), and in southern Colorado. A unique population of A. cyanocarpum was found near Boulder, Colorado, in a pure Pinus ponderosa stand. The closest known infection on its usual host, P. flexilis, is at least 5 miles away and 2000 feet higher (Hawksworth et al. 1975). Tsuga mertensiana is a new host for A. cyanocarpum (Oregon, Knutson and Tinnin 1981). "Pinus aristata," as formerly understood, is now known to comprise two distinct species, Pinus longaeva D. K. Bailey in the Great Basin (California, Utah, and Nevada), and P. aristata sensu stricto in the Rocky Mountains (Colorado, New Mexico, and Arizona (Bailey 1970)). Pinus longaeva is frequently parasitized by A. cyanocarpum in Nevada and Utah; the mistletoe is known only on P. aristata in one locality in southern Colorado.

11. ARCEUTHOBIUM DIVARICATUM. This widespread dwarf mistletoe of the southwestern United States and northern Baja California, Mexico, is still known only on the pinyons. By far its most common hosts are Pinus edulis and P. monophylla. It was previously known also on P. cembroides only in the Davis Mountains of West Texas, and P. quadrifolia in Baja California, but it has since been found on two additional taxa: Pinus discolor in southern New Mexico, and P. edulis var. fallax in Arizona and Utah. Several new locations of the parasite have been found since 1972, but most are within its previously known limits (fig. 6).

12. ARCEUTHOBIUM DOUGLASII. No major range extensions of this widespread parasite of Pseudotsuga menziesii have been reported. See Baranyay and Bauman (1972) for its distribution in British Columbia. New locations found are: the first for coastal British Columbia (near Chilliwack (Van Sickle and Fiddick 1982), and in northwestern Durango, Mexico (Cibrian T. et al. 1980). In 1981, we examined most of the Pseudotsuga stands in Chihuahua but did not discover the parasite there (Hawksworth and Cibrian T. 1984). Cibrian T. et al. (1980) report A. douglasii on Pseudotsuga flahaultii in Durango, but we question whether this tree is distinct from P. menziesii var. glauca. Palmer et al. (1983) report A. douglasii in the Sierra Nevada in Placer County, about 75 miles south of its previously known southern limits in California, but this has not been confirmed. Dooling and Eder (1981) show the known distribution of A. douglasii in Montana.

13. ARCEUTHOBIUM GILLII. This parasite, primarily of Pinus leiophylla var. chihuahuana, is found in northern Mexico and the southwestern United States (fig. 7). New locations for the parasite include: New Mexico (Animas Mountains, Hawksworth and Weiss 1975), Sonora (Ajo Mountains, Mathiasen 1979b), and northern Sinaloa. Pinus cooperi (Chihuahua) is a new host (Hawksworth and Cibrian T. 1984).





Figure 5.--Distribution of *Arceuthobium cyanocarpum*.

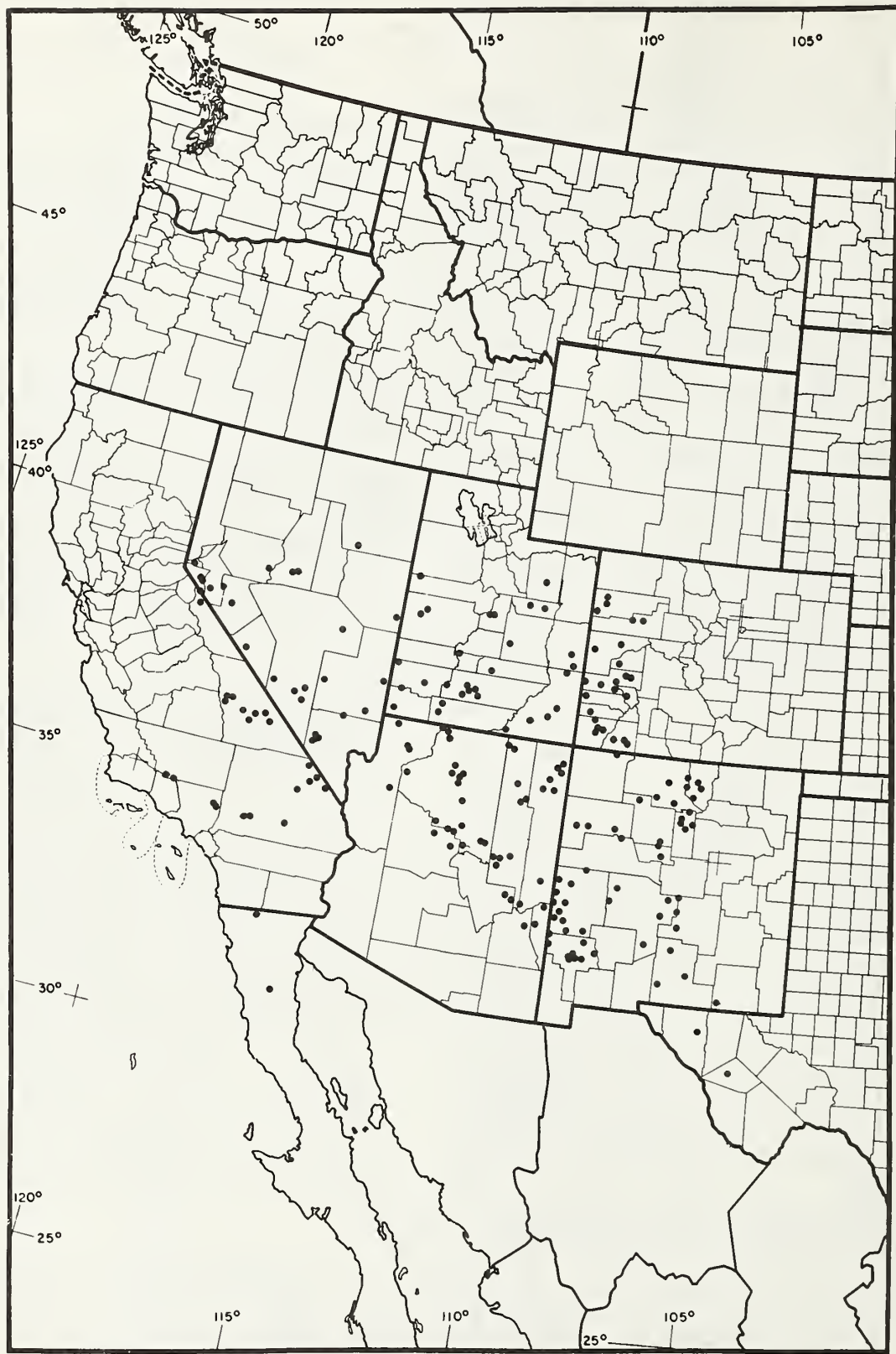


Figure 6.--Distribution of *Arceuthobium divaricatum* in the United States and Mexico.

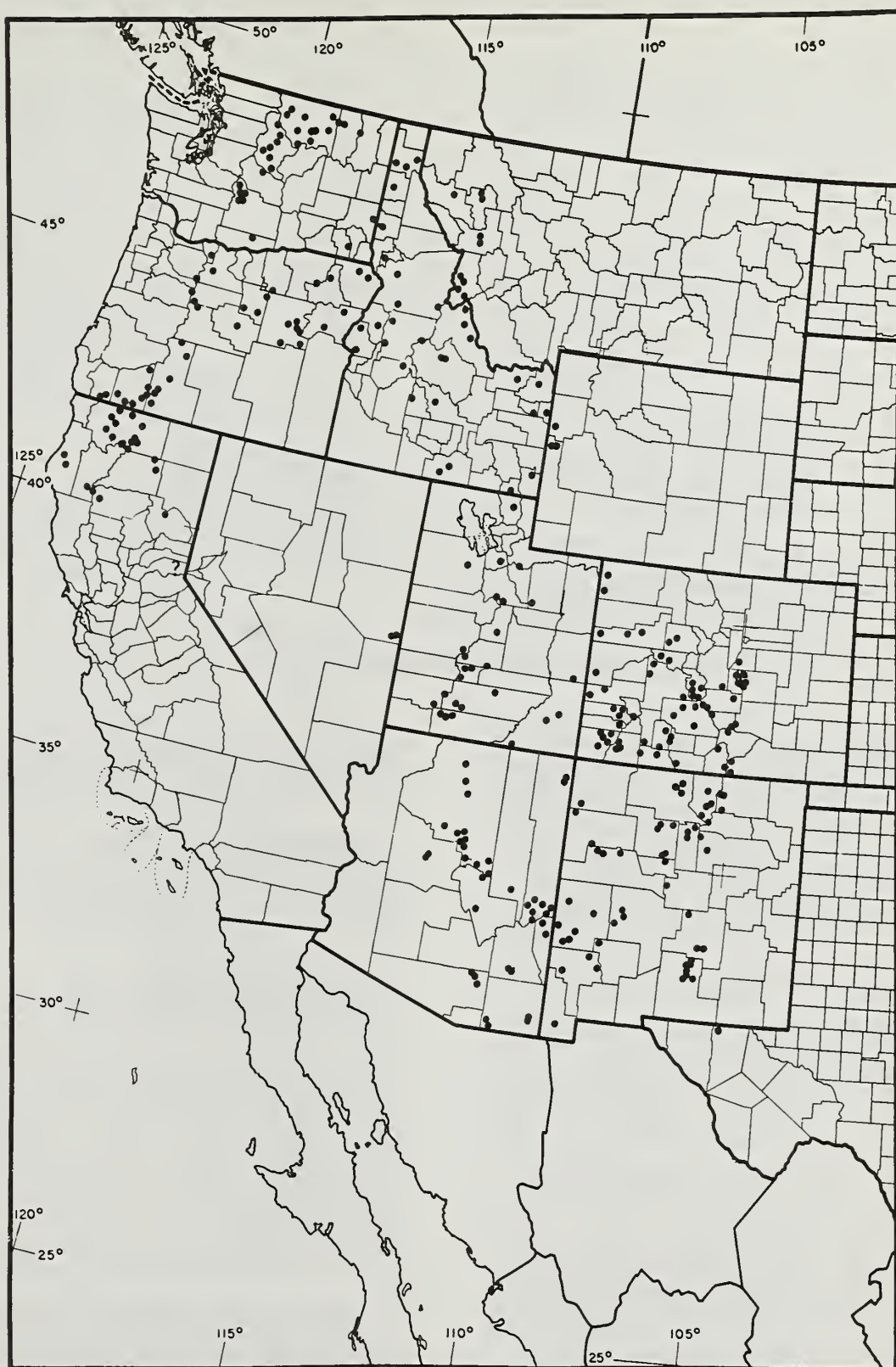


Figure 7.--Distribution of *Arceuthobium douglasii* in the western United States. The report by Palmer et al. (1983) in Placer County, California has not been confirmed.



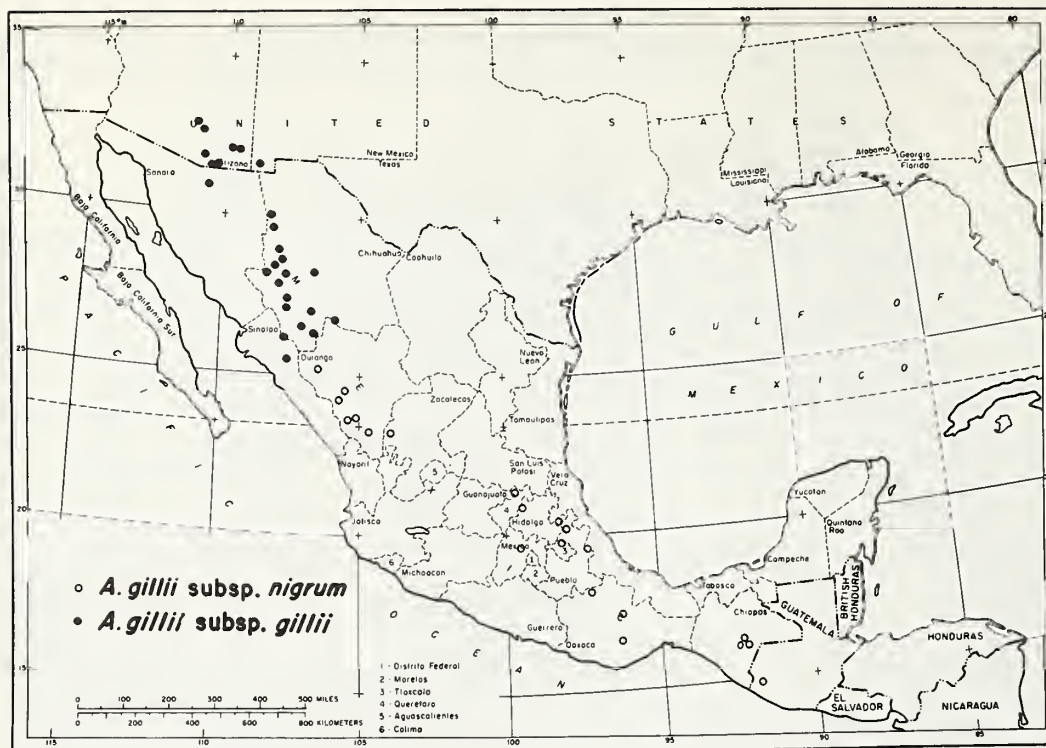


Figure 8.--Distribution of the two subspecies of Arceuthobium gillii in Mexico and the southwestern United States.

14. ARCEUTHOBIUM GILLII subsp. NIGRUM. This parasite of Mexican pines (mainly P. leiophylla var. leiophylla) has been found in several new locations in the States of Queretaro, Zacatecas, Mexico, Puebla, Oaxaca, and Chiapas (fig. 8). New hosts include Pinus oaxacana (Chiapas), P. lawsonii (Oaxaca), P. patula (Chiapas and Puebla), and P. herrerae (Oaxaca), P. patula (Chiapas and Puebla), and P. herrerae (Oaxaca) (Hawksworth and Wiens 1977). We have reports of the taxon in the San Marcos area of Guatemala, but we have seen no collections from that country.

15. ARCEUTHOBIUM GLOBOSUM. This parasite of pines in northern Mexico was reported from Chihuahua and Durango, and has since been found in Sonora (fig. 1). As noted by Hawksworth and Wiens (1977), selection of the type of A. globosum was unfortunate because that population consists of a small plant presently known only from the area around El Salto, Durango on Pinus cooperi. The more common phase of the species is much larger and occurs on other pines (P. arizonica, P. durangensis, and P. engelmannii). Although there are some differences between the small plant composing a single geographically restricted type population and the widespread, larger, rounded form, these do not, at present, appear to be sufficient to warrant taxonomic recognition.

16. ARCEUTHOBIUM GUATEMALENSE. This parasite of

Pinus ayacahuite was described from Guatemala but has since been found in Chiapas and Oaxaca in southern Mexico (Hawksworth and Wiens 1977).

17. ARCEUTHOBIUM HONDURENSE. No new information on this parasite of pines in Honduras has been reported, but it possibly occurs in the Monte Cristo area in northeastern El Salvador (Hawksworth 1982). It is probably the rarest of all the dwarf mistletoes.

18. ARCEUTHOBIUM LARICIS. This is a widespread parasite of Larix occidentalis in Montana, Idaho, Oregon, Washington, and British Columbia. Smith et al. (1972) reported the first known instances in British Columbia of this species on Abies grandis and Pinus ponderosa, which were also rare hosts of it in the U.S. Smith and Wass (1972) showed by inoculations that Abies amabilis is rarely infected by A. laricis. Distribution maps for A. laricis have been published for British Columbia (Baranyay and Bauman 1972), and Montana (Dooling and Eder 1981). A few new localities have been reported in the United States but all are within the previously known range.

19. ARCEUTHOBIUM MICROCARPUM. This species has a highly local distribution, primarily on Picea in Arizona and New Mexico. In the San Francisco Peaks, Arizona, A. microcarpum is common and damaging on Pinus aristata (Mathiasen and Hawksworth 1980). We tentatively classed this population as A. cyanocarpum (Hawksworth and Wiens



1972). Mathiasen (1978) showed in greenhouse tests that P. flexilis is susceptible to A. microcarpum. A major range extension of A. microcarpum is the recent discovery of an isolated population in the Sacramento Mountains in southern New Mexico, some 270 km east of its previously known limits in the Mogollon Mountains (Mathiasen and Jones 1983).

20. ARCEUTHOBIUM OCCIDENTALE. This species is confined to California where it is found on a number of hard pines. Its principal hosts are Pinus sabinana, P. radiata, and P. muricata; it sometimes parasitizes P. coulteri, P. attenuata, and P. contorta subsp. bolanderi. Contrary to reports in our 1972 monograph, the species does infect P. ponderosa, at least in the Santa Lucia Mountains. Possibly A. occidentale may also parasitize Pinus jeffreyi on the San Benito Mountains where a few infected Jeffrey pines were observed in infected P. sabiniana-P. coulteri stands (Griffin 1975). A few new locations for A. occidentale have been reported, but all are within the general range previously reported. The taxonomic status of this species and its relation to A. campylopodum are discussed in the "Taxonomic Problems" section of this paper.

21. ARCEUTHOBIUM PUSILLUM. No major changes in the hosts or geographic distribution of this species have been reported. However, our observations in New Hampshire suggest that Picea rubens should be considered a principal host because A. pusillum is widespread in pure stands of this tree (Hawsworth and Shigo 1980). A. pusillum was also found in a Pinus banksiana stand near Isabella, Minnesota (Baker et al. 1978). Coulombe (1973) reported Abies balsamea as a new host for A. pusillum in St. Jean, Quebec. The tree was transplanted from a bog in the Farnham region and probably became infected there. Distribution maps of A. pusillum have been published for Quebec (Lavallee 1973), Ontario (Myren and Gross 1977), and Newfoundland (Singh 1982). A survey in the Lower Peninsula of Michigan showed the mistletoe to be quite local (Mathiasen 1979).

22. ARCEUTHOBIUM RUBRUM. This rare Mexican parasite of pines was previously known only in local areas in the Sierra Madre Occidental of Durango and Sinaloa. Since then, a disjunct population nearly 1200 km southeast from the Sierra Madre populations was found in southern Oaxaca by R. S. Peterson (Hawsworth and Wiens 1977). In Oaxaca, the principal hosts are Pinus lawsonii, P. michoacana, and P. pseudostrobus; P. oaxacana is occasionally parasitized. In Durango, the mistletoe parasitizes P. cooperi, P. durangensis, P. engelmanni, P. teocote, and P. herrerae, but none of these pines occur at the Oaxacan site. The status of the southern population deserves further study.

23. ARCEUTHOBIUM STRICTUM. This very distinctive, but rare, species on pines is still known only from a few locations in Durango, Mexico.

Player (1979) investigated its pollination ecology.

24. ARCEUTHOBIUM TSUGENSE. This common parasite of Tsuga heterophylla ranges from near Haines, Alaska to northern California. A distribution map for British Columbia was published by Baranyay and Bauman (1972). It is rare on Tsuga mertensiana in Alaska and British Columbia (Shaw 1982, Van Sickle and Fiddick 1982), but this is its most frequent host in the mountains of Oregon and California. Inoculation studies with Pseudotsuga menziesii indicated that the species could be rarely infected by A. tsugense (Smith and Wass 1972), and also P. menziesii has recently been reported to be a rare, natural host in British Columbia (Hunt and Smith 1978). The status of the race of this mistletoe on shore pine (Pinus contorta subsp. contorta) is still in question. Extensive populations of A. tsugense occur on this pine from the San Juan Islands, Washington, along the eastern shore of Vancouver Island, and coastal British Columbia (Smith 1974, Smith and Wass 1976, Wass 1976).

25. ARCEUTHOBIUM VAGINATUM subsp. VAGINATUM. This large, widespread Mexican species attacks at least 12 species of pines, and ranges from west-central Chihuahua to northern Oaxaca, and north in the Sierra Madre Oriental to southern Coahuila (fig. 9). It has been found for the first time in Sinaloa.

26. ARCEUTHOBIUM VAGINATUM subsp. CRYPTOPODUM. This common and damaging parasite, mainly of Pinus ponderosa var. scopulorum, ranges from northern Colorado and central Utah to Chihuahua and Coahuila, Mexico (fig. 9). Several new locations for the parasite have been found but most of them, except in Coahuila, are within the previously known range of the taxon. New hosts for this subspecies are Pinus cooperi and P. durangensis in Chihuahua (Hawsworth and Cibrian T. 1984).

27. ARCEUTHOBIUM VAGINATUM subsp. DURANGENSE. This local taxon is known mainly from along the Durango-Mazatlan highway in western Durango and adjacent parts of Sinaloa (Hawsworth and Wiens 1977) (fig. 9). It was recently collected near Cosala, Sinaloa, about 150 km to the north of its previously known limits (collected by R. L. Mathiasen, specimen at PFF).

28. ARCEUTHOBIUM VERTICILLIFLORUM. This primitive Mexican species is the most distinctive dwarf mistletoe. It is characterized by its large fruits (over 1 cm long) and its verticillate staminate flowers. It was previously known only from the vicinity of El Salto, Durango, but a second population was recently discovered at El Huacal, Durango, about 200 km to the north (Cibrian T., et al. 1980).

Old World

29. ARCEUTHOBIUM CHINENSE. This small species parasitizes Keteleeria in southwestern China (Yunnan and Sichuan provinces). The occurrence



Figure 9.--Distribution of the three subspecies of Arceuthobium vaginatum in the United States and Mexico. Only the southern United States collections of subsp. cryptopodum are shown.

of 4-parted staminate flowers distinguishes this species from other Chinese taxa, which are primarily 3-parted. The only known distribution map for A. chinense was recently published by Kiu and Ren (1982). Kiu (these proceedings) notes that reports of this species on Abies are in error and probably based on misidentification.

30. ARCEUTHOBIUM MINUTISSIMUM. This species is essentially restricted to Pinus wallichiana, a high altitude pine of the Himalayas. It occurs in northern Tibet.<sup>5</sup> This very small species (with shoots only about 5 mm high) was thought to be exclusively parasitic on Pinus wallichiana. However, Pinus gerardiana was recently found to be a rare host of this mistletoe in areas where this tree occurs in mixed stands with Pinus wallichiana in Pakistan (Zakaulah and Badshaw 1984). Rechinger (1976) reports it on Cedrus deodara in Pakistan, but this record has not been confirmed. The recent report of A. minutissimum at low elevations in southern India on an angiosperm (Gaur 1981) is in error and was based on a fungus that superficially resembles the dwarf mistletoe.

31. ARCEUTHOBIUM PINI. This parasite of pines in southwestern China was previously known from Yunnan and Sichuan provinces. It was recently found in Tibet by Kiu and Ren (1982), who present the only known distribution map for this species.

32. ARCEUTHOBIUM OXYCEDRI. This parasite of junipers has a vast range of over 9,000 km from

Spain throughout the Middle East, and to Pakistan, India, and Tibet (Hawksworth and Wiens 1976, Kiu and Ren 1982). Hawksworth and Wiens (1976) list it on 10 species of junipers, and Lararev and Girgorov (1980) record it on 3 additional species in the Crimea, USSR. They also found it on introduced Juniperus thuifera. Kiu (these proceedings) lists it on two new hosts: Sabina (Juniperus) wallichiana and S. tibetense in Tibet.

#### SYNOPSIS OF THE GENUS

As currently recognized, the genus Arceuthobium comprises 41 taxa, 33 in the New World, and 8 in the Old (Table 1). The countries with the greatest increase since 1972 are China (2 to 6 taxa), Mexico (16 to 19), and Guatemala (2 to 5). Since 1972, Arceuthobium has been first reported in El Salvador and Cuba (assuming that A. cubense is, in fact, a dwarf mistletoe).

#### TAXONOMIC PROBLEMS IN ARCEUTHOBIUM

Although we feel that we have the "big picture" of the genus Arceuthobium in the world there are a number of taxonomic questions that remain to be resolved.

1. For some time, it has been recognized that the populations of Arceuthobium tsugense on shore pine (Pinus contorta subsp. contorta) may be distinct from those on Tsuga heterophylla. Populations on Pinus rarely infect Tsuga and vice versa (Smith and Wass 1976). Taxonomic studies of the two races, or pathotypes, are underway. Perhaps they should be treated as forma speciales (as we have done for A. abietinum on

<sup>5</sup>Personal communication, Chen Mo-me, forest pathologist, Academy of Forestry, Beijing, 1982.



Abies concolor and A. magnifica), or as subspecies, if consistent morphological differences can be found. The populations on Tsuga mertensiana and white pines in southern Oregon and California may also be distinct.

2. The Arceuthobium campylopodum-A. occidentale alliance is more complex than we originally thought. For example, in the monograph we listed Pinus ponderosa, a primary host of A. campylopodum, as immune to A. occidentale. This is not the case, since A. occidentale is locally common on P. ponderosa as well as P. coulteri in the Santa Lucia Mountains in California. Branching patterns of A. occidentale and A. campylopodum are distinct (Mark and Hawksworth 1982). A series of cross-inoculation studies of A. occidentale and A. campylopodum conducted by W. Mark and R. Scharpf on several pines in various parts of California should shed light on the taxonomic relationships of these two economically important taxa.

3. The status of several Mexican taxa, particularly the subspecies of A. globosum, A. gillii, and A. vaginatum, and the two populations of A. rubrum, should be re-evaluated. Some of these may warrant specific status, as the differences between them are at least as great as between several recognized species in section Campylopoda.

4. The status of the recently described A. cubense in Cuba should be confirmed. If this turns out to be a valid Arceuthobium on a member of the Podocarpaceae, we may have to re-evaluate our speculation that the genus arose in eastern Asia as a parasite of pines.

5. Now that eight members of the genus are known from the Old World, it would be worthwhile to attempt to develop supra-specific categories for them.

6. Incorporation of data from new taxonomic approaches, such as isozyme electrophoresis, should be useful in helping understand the taxonomy and evolutionary relationships of the dwarf mistletoes (see papers by Nickrent et al. and Linhart, these proceedings).

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# Arceuthobium and its Hosts in Southwestern China<sup>1</sup>

Kiu Hua-shing<sup>2</sup>

**Abstract.**--Arceuthobium is represented in Southwestern China by five taxa, two of which were recently described: A. tibetense H. S. Kiu et W. Ren (1982) on Abies in Xizang, and A. pini var. sichuanense H. S. Kiu (1984) on Picea in Xizang and Sichuan. The other three known taxa are A. chinense on Keteleeria in Sichuan and Yunnan; A. pini on Pinus in Xizang, Sichuan, and Yunnan; and A. oxycedri on Sabina in Xizang. Reports of A. chinense on Abies are questioned as the mistletoe is not known within the elevational range of Abies.

## INTRODUCTION

The Chinese name of Arceuthobium M. Bieb. is You-shan-ji-sheng Shu. It is widely distributed in Southwestern China and is represented by 5 taxa: 4 are found in Xizang (Tibet) Autonomous Region, 3 in Sichuan Province, and 2 in Yunnan Province (Table 1). Their known elevational range is from 1,500 to 4,100 meters.

Table 1.--Distribution of Arceuthobium in Southwestern China

Taxon	Xizang	Sichuan	Yunnan
<u>A. chinense</u>	----	X	X
<u>A. pini</u>	X	X	X
<u>A. pini</u> var. <u>sichuanense</u>	X	X	----
<u>A. tibetense</u>	X	----	----
<u>A. oxycedri</u>	X	----	----
Number of taxa	4	3	2

Note: (----) indicates not known in the Province (Region).

The paper is based on examining specimens collected by the Chinese botanists through many expeditions during the last fifty years, and those of my field work in recent years.

Biologic characteristics presented for each of the 5 taxa include: diagnosis, hosts; (Table 2), and others.

<sup>1</sup>Paper presented at the Symposium on the Biology of Dwarf Mistletoes, in conjunction with the A.I.B.S. Meetings, August 8, 1984, Fort Collins, Colorado.

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Table 2.--Hosts of Chinese dwarf mistletoes

<u>Arceuthobium</u>	Principal host	Secondary host
<u>A. chinense</u>	<u>Keteleeria evelyniana</u>	
<u>A. pini</u>	<u>Pinus densata</u>	<u>Pinus yunnanensis</u>
<u>A. pini</u> var. <u>sichuanense</u>	<u>Picea likiangensis</u> var. <u>balfouriana</u>	
<u>A. tibetense</u>	<u>Abies georgei</u>	
<u>A. oxycedri</u>	<u>Sabina wallichiana</u> <u>Sabina tibetica</u>	

1. Arceuthobium chinense Lecomte (1915). (Keteleeria dwarf mistletoe).

Plants 2-8 (10-12) cm. high, yellowish-green or green; branches opposite, rarely 3-4 (-6) verticillate; basal diameter of dominant shoots 1-2 mm. Flowers axillary or terminal; male flower ca. 2 mm. in diameter, perianth segments 4, lobes subtriangular, ca. 1.5 mm. long; female flower greenish, 1 mm. long. Mature fruit ovoid, 4-6 mm. long and 3-4 mm. wide, proximal portion yellowish-green or glaucous. Flowering period: July to October. A. chinense induces systemic witches' brooms on Keteleeria evelyniana Mast., which is its only actual host. In central and northwestern Yunnan and southwestern Sichuan at elevational range of 1,500 to 2,700 m.

According to M. H. Lecomte's (1915) original description: this dwarf mistletoe is parasitic on the Abies and the type specimen of A. chinense (Delavay s.n.) (n.v.) was collected at Ta-pin-tze in Yunnan. But this locality is only 2,470 m. above sea level; no species of Abies has been found in such low altitude. So, its possible that M. H. Lecomte misidentified the host plant.

2. Arceuthobium pini Hawsworth et Wiens (1970). (Alpine pine dwarf mistletoe)

Plants 5-15 (-20) cm. high, greenish-yellow or greenish; branches opposite, rarely 3-4 verticillate; basal diameter of dominant shoots 1.5-2.5 mm. Male flowers 1 or 2 terminal on short branches, 2-2.5 mm. in diameter, perianth segments 3, rarely 4, lobes 1-1.5 mm. long. Female flowers terminal or axillary, ca. 1 mm. long. Mature fruit ellipsoid, 3-4 mm. long and 2-2.5 mm. wide, proximal portion greenish-yellow. Flowering period: April to June. It is known to be parasitic on Pinus densata Mast. and P. yunnanensis Fr.; these infected hosts did not form witches' brooms. In eastern Xizang, northwestern Yunnan, and southeastern Sichuan at elevational range of 2,700-3,500 (-4,000) m.

2a. Arceuthobium pini Hawsworth et Wiens var. sichuanense H. S. Kiu in Acta Phytotax. Sinica 22(3): 1984.

Plants 2-6 cm. high, greenish-yellow; basal diameter of dominant shoots 1-1.5 mm. Flowers axillary or terminal on short branches; male flower 1.5-2 mm. in diameter, perianth segments 3. Mature fruit ellipsoid, 3-4 mm. long and 1.5-2 mm. wide. Flowering period: June to July. Parasitic on Picea likiangensis (Fr.) Pritz. var. balfouriana (Rehd. et Wils.) Hallier ex Slavin; host induced to systemic witches' brooms. In eastern Xizang and western Sichuan. Known elevational range is 3,800 to 4,100 m.

Specimens examined: Sichuan: Dawu, in 1974, Y. T. Wu et B. C. Gao 111629, ♂ (type CDBI). Xizang: Biru, in 1976, T. T. Dao 11287, ♀ (KUN).

3. Arceuthobium tibetense H. S. Kiu et W. Ren (1982).

Plants 0.5-2.2 cm. high, yellowish-green, branches opposite; basal diameter of dominant shoots ca. 1.5 mm. Flowers terminal or axillary; male flower 2 mm. in diameter, perianth segments 3, rarely 4, lobes subtriangular, ca. 1.2 mm. long. Fruit ellipsoid, ca. 2 mm. long, proximal portion glaucous. Flowering period: May to June. Parasitic on Abies georgei Orr and induces witches' brooms. In eastern Xizang at an elevation ca. 3,400 m. in fir forest.

Specimens examined: Xizang: Mainling, in 1981, K. Z. Chao 14, ♂ (SCBI), ibidem, K. Z. Chao 14 A, ♀ (SCBI).

4. Arceuthobium oxycedri (DC.) M. Bieb. (1819).

Plants 5-16 cm. high, greenish-yellow; branches opposite, rarely 4-6 verticillate; basal diameter of shoots 1.5-3 (-4) mm. Male flowers solitary or 2-3 terminal on short branches, ca. 2 mm. in diameter, perianth segments 3, sometimes 4. Female flowers terminal or axillary. Fruit ellipsoid, ca. 3 mm. long.

Flowering period: August to September. Parasitic on Sabina wallichiana (Hook. f. et Thoms.) Kom. and S. tibetica Kom. In eastern Xizang at elevational range of 3,000 to 3,500 m.

Specimens examined: Xizang: Riwoqe, in 1952, P. C. Tsoong 5319 (PE); Bomi, in 1965, T. S. Ying et al. 1984 (PE). These specimens might be a geographic variation of the widespread species A. oxycedri.

Key to the dwarf mistletoes in southwestern China (Kiu and Ren 1982).

1. Staminate sepals 4; basal diameter of dominant shoots 1-2 mm.; plants 2-8 (12) cm. high; side branches frequently less than 1 cm. long; parasitic on Keteleeria (Yunnan, Sichuan). . . . .

. . . . . A. chinense Lecomte

1. Staminate sepals 3, rarely 4.

2. Parasites of Pinaceae

3. Plants 5-20 cm. high; basal diameter of dominant shoots 1.5-2.5 mm.; side branches longer than 1 cm., staminate flowers 2-2.5 mm. wide; sepals 3 rarely 4; parasitic on Pinus (Yunnan, Sichuan, Xizang) . . . . . A. pini Hawsworth et Wiens

3. Plants frequently less than 5 cm. high, basal diameter of dominant shoots 1-1.5 mm. wide.

4. Parasitic on Picea; plants 2-6 cm. high; side branches about 1 cm. long, staminate flowers 1.5-2 mm. wide; sepals 3 (Sichuan, Xizang). . . . . A. pini var. sichuanense H. S. Kiu

4. Parasitic on Abies; plants 0.5-1.7 (2.2) cm. high; side branches 3-6 mm. long; staminate flowers 2 mm. wide; sepals 3, rarely 4 (Xizang). . . . . A. tibetense H. Kiu et W. Ren

2. Parasites of Cupressaceae (Sabina), plants 5-16 cm. high; staminate flowers 2 mm. diameter; sepals 3, rarely 4 (Xizang, . . . . . A. oxycedri (DC.) M. Bieb.

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# Biosystematic and Evolutionary Relationships among Selected Taxa of *Arceuthobium*<sup>1</sup>

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Abstract: Genetic relationships within 19 taxa of New World *Arceuthobium* were examined using starch gel electrophoresis of seed endosperm. Allelic frequency data for 11 polymorphic loci from forty populations of the above taxa were compared using unweighted pair group cluster analysis and Nei's genetic identity. *Arceuthobium americanum* segregates first from the remaining taxa which agrees with the previously generated phenogram (Hawksworth and Wiens 1972) based on morphological, physiological, ecological, and host data. *A. douglasii* and *A. pusillum* are not shown to be closely related nor recent derivatives of the *Campylopodium* stock. The former taxon shows affinity with "Section *vaginata*," here including *A. vaginatum* ssp. *cryptopodium*, *A. vaginatum* ssp. *durangense*, *A. gillii*, and *A. divaricatum*. A Mexican taxon, *A. rubrum*, although somewhat transitional, groups more naturally with "Section *vaginata*" than with the *Campylopodium* group. Within this group, eleven taxa show high levels of genetic similarity (80% or greater) making species delimitations more difficult. Owing to morphological reductions and phenotypic convergence, phylogenetic relationships in these parasites have been obscured.

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## INTRODUCTION

The genus *Arceuthobium* is currently recognized as comprising 32 North American taxa with centers of species diversity in Northern California and the Sierras of Mexico (Hawksworth and Wiens 1972; 1977). The aim of this study was to obtain new sources of evidence to address taxonomic and evolutionary questions in 19 New World taxa of dwarf mistletoes.

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Electrophoresis has proven to be a powerful tool in delimiting plant and animal taxa at the generic and specific level. The advantages and shortcomings of this technique have been discussed (Avisé 1974; Gottlieb, 1977). This method of analysis was chosen primarily because it allows a sensitive assay of gene products (proteins and/or enzymes) across many gene loci and can be readily applied to numerous individuals sampled from natural populations. Crawford (1983) presents a convincing discussion of the value of this method over other chemosystematic approaches.

In parasitic plants such as *Arceuthobium*, reductions and convergences tend to obscure phylogenetic relationships (as measured by traditional morphological analyses) by limiting the number of characters, both vegetative and reproductive, available for study. The chances for "errors in assignment" (Kuijt 1969) thus become enormous. The taxonomy and classification of dwarf mistletoes has been the source of varied interpretations (Gill 1935; Kuijt 1955; Hawksworth and

Wiens 1972), yet none of these studies utilized biosystematic approaches involving crossing experiments or genetic analysis at the macromolecular level. For this reason electrophoresis seemed especially amenable to this taxonomically difficult group.

In contrast to the early work by Gill (1935), Hawksworth and Wiens (1972) do not delimit dwarf mistletoe taxa solely on the basis of host relationships. A major tenet of their system is that the mistletoes "maintain morphological integrity on separate hosts and are identifiable". Such a system is clearly an advancement over the previous one. For example, both *Arceuthobium vaginatum* ssp. *cryptopodum* and *A. cyanocarpum* may parasitize *pinus ponderosa*, yet these two taxa are quite distinct morphologically.

The relevance of the results of this study can be better understood given an initial introduction to the results of the phenetic analysis conducted by Hawksworth and Wiens (1972). In that study, characters involving morphology, physiology, host associations, and secondary compound chemistry were tabulated for 28 North American taxa and subjected to a Graph Theory Model of clustering (Wirth et al. 1966; Irwin and

Rogers 1967). The resulting dendrogram is reproduced in Figure 1. A summation of the phylogeny of the genus proposed by Hawksworth and Wiens (1972) is shown in Figure 2. Three letter abbreviations for dwarf mistletoe taxa shown in Figure 2 are explained in Table 1.

The 19 taxa utilized in this study are arranged according to the above taxonomic hierarchy of Hawksworth and Wiens (1972) and Kuijt (1955) as shown in Table 1. Hawksworth and Wiens (1972) proposed two subgenera, (*Arceuthobium* and *Vaginata*, based primarily on the presence of verticillate secondary branching (the former subgenus) or flabellate secondary branching (the latter subgenus). Three sections within Subgenus *Arceuthobium* were also established (*vaginata*, *Campylopoda*, and *Minuta*). Section *vaginata* is considered to be the most primitive group whereas Section *Minuta* the most advanced owing to its diminutive shoot size and the occurrence of isophasic broom formation.

A particularly troublesome group within the genus, however, involves 13 taxa in Section *Campylopoda*, Series *Campylopoda* (as defined by Hawksworth and Wiens 1972). Nine of the 13 taxa were treated as forms of one variable species, *Arceuthobium campylopodum*, by Kuijt (1955)

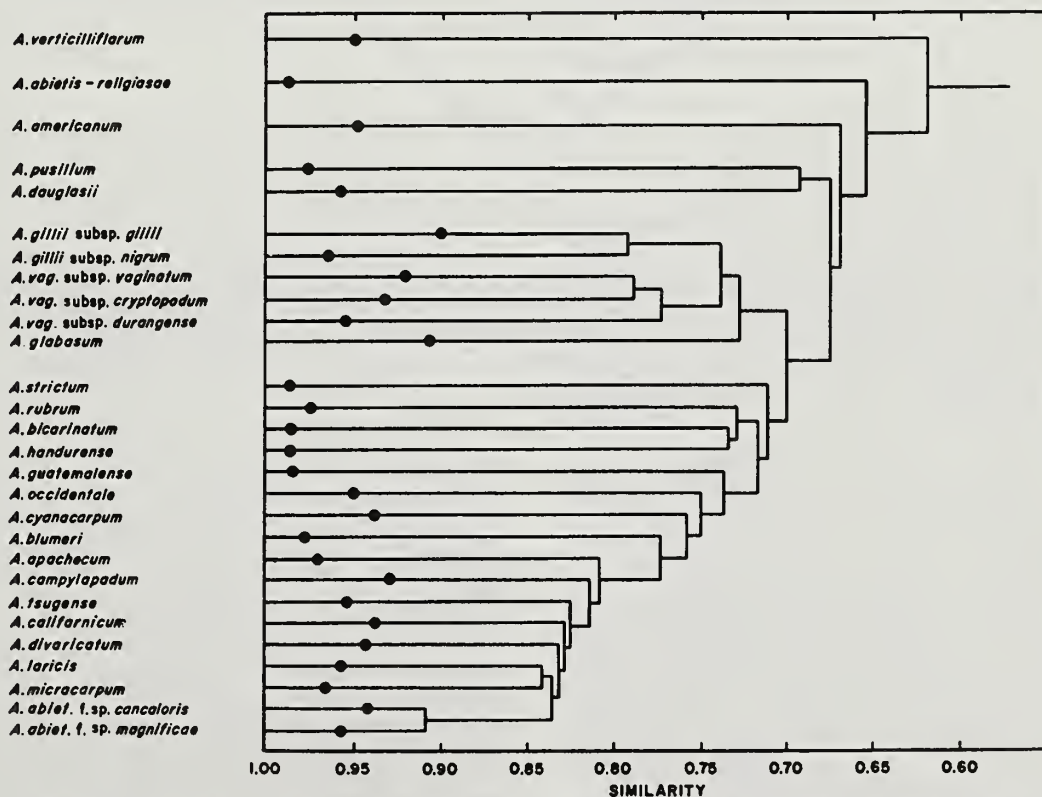


Figure 1.--Dendrogram showing the results of taximetric analyses of most New World dwarf mistletoes (reproduced from Hawksworth Wiens, 1972). The scale shows overall phenetic similarity based on combined analysis of 60 morphological, physiological, and host characters. The dot shows the level of intrataxon similarity. The further the dot from 1.0, the more variable the taxon.

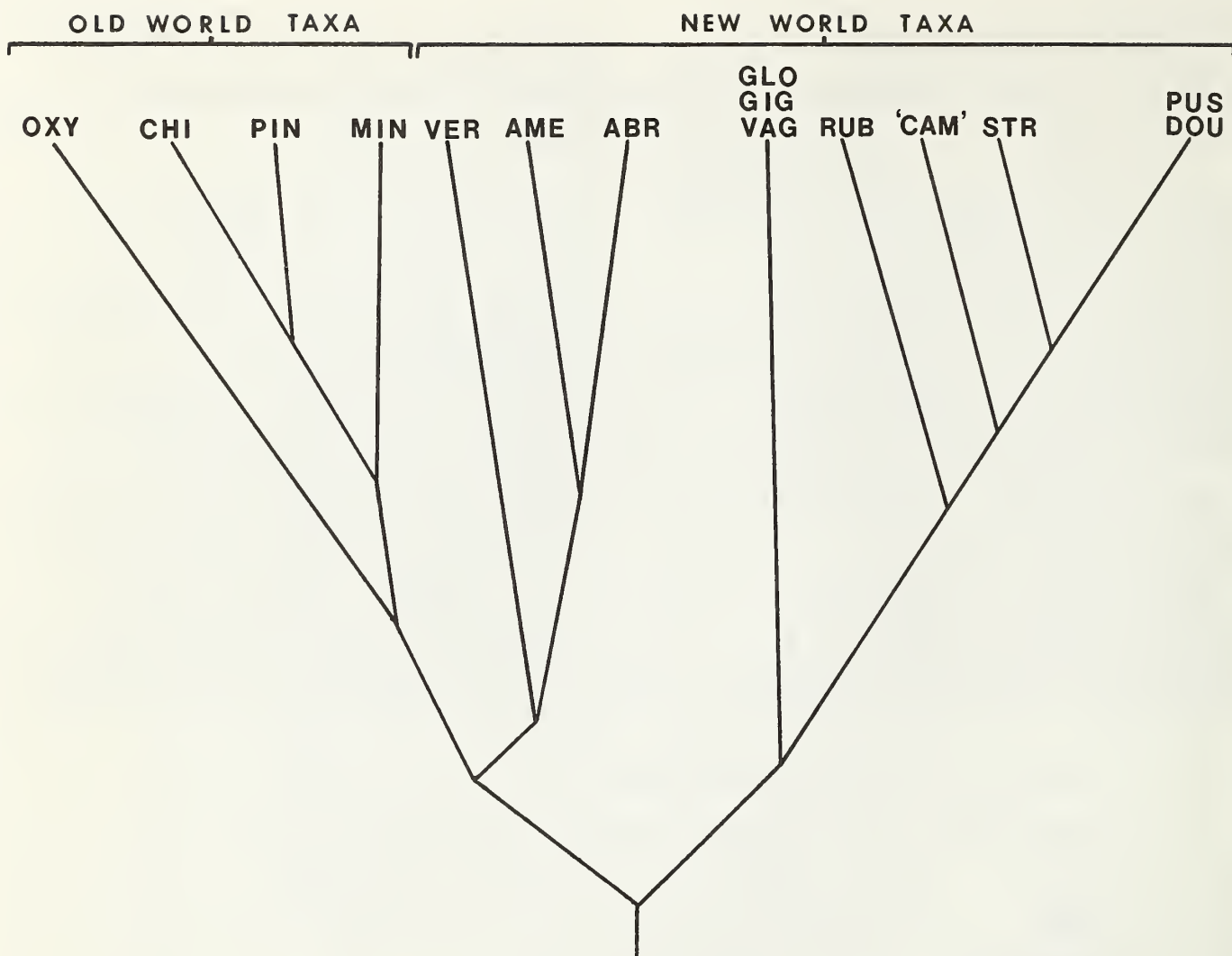


Figure 2.--presumed phyletic relationships among Old and New World Arceuthobium, redrawn from Hawksworth and Wiens (1972). New World taxon abbreviations not listed in Table 1 are: VER = A. verticilliflorum, ABR = A. abietis-religiosae. Old World taxa are: OXY = A. oxycedri, CHI = A. chinense, PIN = A. pini, and MIN = A. minutissimum.

(Table 1). Kuijt (1973) questioned whether the observed discontinuities warranted segregation at the specific level (as in the current system) or whether they would be best incorporated within an ecotype concept. The major discrepancy between the two systems involves the specific status of members of a large group (13 taxa) related to the ponderosa pine dwarf mistletoe Arceuthobium campylopodum. In view of the overall morphological similarity of the taxa in question, relationships can only be addressed by determining whether there is gene flow between these taxa in the natural, sympatric populations.

The degree of sympatry among dwarf mistletoes is often quite high. In areas such as the Sierra Nevada of Northern California, as many as seven taxa can be

found within "gene exchange" distances. Arceuthobium is dioecious, therefore an obligate outcrosser. pollination appears to be mediated by both wind and (possibly primarily) insect vectors (penfield et al. 1976; Gilbert and punter, these proceedings). Anthesis is both temporally and spatially overlapping in many taxa, yet there exists no report of hybridization or polyploidy in the genus (Hawksworth and Wiens 1970; 1972). Unfortunately all taxa in the genus have  $X=14$  as a base chromosome number (Wiens. 1968), hence detection of hybrids utilizing cytology would be extremely difficult. With reference to Arceuthobium campylopodum and A. occidentale, Hawksworth and Wiens (1970) state "if mutual pollinating agents are available,



Table 1.-- Subgeneric classification of New World dwarf mistletoes used in this study according to Hawksworth and Wiens (1972) compared to Kuijt (1955)

Hawksworth and Wiens (1972)	Kuijt (1955)
I. Subgenus <u>Arceuthobium</u> No Sections Designated <u>A. americanum</u> (AME)	<u>A. americanum</u>
II. Subgenus <u>Vaginata</u> A. Section <u>Vaginata</u> <u>A. gillii</u> (GIG) <u>A. vaginatum</u> ssp. <u>cryptopodum</u> (VAC) <u>A. vaginatum</u> ssp. <u>durangense</u> (VAD)	N.C. <u>A. vaginatum</u> N.C.
B. Section <u>Campylopoda</u> 1. Series <u>Campylopoda</u> <u>A. abietinum</u> f. sp. <u>concoloris</u> (ABC) <u>A. abietinum</u> f. sp. <u>magnificae</u> (ABM) <u>A. apachecum</u> (APA) <u>A. blumeri</u> (BLU) <u>A. californicum</u> (CAL) <u>A. campylopodum</u> (CAM) <u>A. cyanocarpum</u> (CYA) <u>A. divaricatum</u> (DIV) <u>A. laricis</u> (LAR) <u>A. microcarpum</u> (MIC) <u>A. occidentale</u> (OCC) <u>A. tsugense</u> (TSU)	<u>A. campylopodum</u> f. <u>abietinum</u> <u>A. campylopodum</u> f. <u>abietinum</u> N.C. N.C. N.C. <u>A. campylopodum</u> f. <u>campylopodum</u> <u>A. campylopodum</u> f. <u>cyanocarpum</u> <u>A. campylopodum</u> f. <u>divaricatum</u> <u>A. campylopodum</u> f. <u>laricis</u> <u>A. campylopodum</u> f. <u>microcarpum</u> <u>A. campylopodum</u> f. <u>campylopodum</u> <u>A. campylopodum</u> f. <u>tsugensis</u>
2. Series <u>Rubra</u> <u>A. rubrum</u> (RUB)	N.C.
C. Section <u>Minuta</u> <u>A. douglasii</u> (DOU) <u>A. pusillum</u> (PUS)	<u>A. douglasii</u> <u>A. pusillum</u>

N.C. = Not Cited

genes should be exchanged if the plants are crossable."

Early in this study it was decided that "seeds" (used in the functional sense since members of the viscaceae lack true ovules) were the most practical tissue for use. Arceuthobium seeds are composed mainly of triploid endosperm (Bhandari and Nanda 1968) and have no true seed coat. Seeds are ideal for electrophoretic analysis because they 1) are easy to collect and transport in quantity, 2) can be stored for reasonably long periods of time under the proper conditions (Knutson 1971; Wicker 1974), 3) gave good enzyme activity in preliminary gel runs, and 4) can be harvested for analysis at the same developmental stage, thereby controlling "ontogenetic" enzyme variation (Scandalios 1974). In addition, seeds probably contain fewer secondary compounds than shoots. These compounds have been shown to be present in quantity in dwarf mistletoe shoots (Crawford and Hawksworth 1979) and are known to interfere with enzyme activity (Kelly and Adams 1977; Mitton, et al. 1979). Since the inception of the present study, Linhart (these proceedings) has successfully utilized dwarf mistletoe shoot material.

## MATERIALS AND METHODS

Herbarium voucher specimens and seed material of 19 taxa of dwarf mistletoe were obtained in the fall of 1981 and 1982. Pressed specimens were deposited at the Willard S. Turrell Herbarium at Miami University (MU). Although 24 of the 32 North American dwarf mistletoe taxa were observed in the field, not all had ripe fruits at the time of visitation, thus precluding their inclusion in the electrophoretic analysis. Seed viability was highest when collected during the "normal" period of dehiscence.

Table 2 lists the 40 populations utilized in this study with information on hosts and exact geographic location. The three letter abbreviations for each dwarf mistletoe taxon corresponds to the names shown in Table 1. These abbreviations will be used throughout the remainder of this paper. The full names of the host species (listed by numbers in Table 2) are given in Table 3.

All seeds were collected from dehiscing shoots into paper bags and stored in plastic bags at 2-4°C at ca. 80% relative humidity. Seeds from single female mistletoe shoots were collected in separate bags and attempts were made to obtain samples from 20 randomly selected

Table 2.-- Collection information for 40 populations of Arceuthobium used in this isozyme study.

Col. #	Taxon Abbr.	Host #*	Locality Pop. Name, Co., State	Township, Range, Section
1.	1917 ABC	1	Cabbage patch, Calaveras, CA	T 7N R17E Sec. 30
2.	1940 ABC	1	Sumerhaven, pima, AZ	T12S R15E Sec. 1
3.	1906 ABM	2	Greenhorn, Kern, CA	T24S R31E Sec. 36
4.	1910 ABM	2	Bass Lake, Madera, CA	T 6S R23E Sec. 18
5.	1916 ABM	2	Cabbage patch, Calaveras, CA	T 7N R17E Sec. 30
6.	1933 ABM	2	Bucks Lake, plumas, CA	T23N R 6E Sec. 11
7.	1939 APA	19	Bear Wallow, pima, AZ	T12S R16E Sec. 5
8.	1948 APA	19	Hospital Flat, Graham, AZ	T 9S R24E Sec. 10
9.	1945 APA	19	Mt. Wrightson, Santa Cruz, AZ	T20S R14E Sec. 13
10.	1951 APA	19	Three Forks, Apache, AZ	T 6N R28E Sec. 34
11.	1937 BLU	19	Carr Canyon, Cochise, AZ	T23S R19E Sec. 1
12.	1930 CAL	13	Quincy, Plumas, CA	T24N R10E Sec. 33
13.	1924 CAM	16	Emerald Bay, El Dorado, CA	T13N R17E Sec. 22
14.	1742 CAM	12	Forest, Sierra, CA	T19N R10E Sec. 19
15.	1750 CAM	16	Providence, plumas, CA	T25N R 8E Sec. 11
16.	1958 CYA	11	Lake Sabrina, Inyo, CA	T 8S R31E Sec. 29
17.	1973 CYA	11	Pingree-2, Larimer, CO	T 7N R73W Sec. 15
18.	1953 DIV	10	Red Hill, Greenlee, AZ	T 4N R31E Sec. 22
19.	1801 LAR	3	Malheur, Grant, OR	T15S R31E Sec. 15
20.	1950 MIC	7	Three Forks, Apache, AZ	T 6N R28E Sec. 34
21.	1947 MIC	4	Hospital Flat, Graham, AZ	T 9S R24E Sec. 3
22.	1962 OCC	18	Placerville, El Dorado, CA	T10N R11E Sec. 11
23.	1853 RUB	20	El Salto, Durango, Mexico	---- ---- ---- --
24.	1927 TSU	15	Mt. Elwell, plumas, CA	T21N R12E Sec. 7
25.	1932 AME	8	Bucks Lake, plumas, CA	T23N R 7E Sec. 3
26.	1929 AME	8	Mt. Elwell, plumas, CA	T21N R12E Sec. 7
27.	1925 AME	8	Lake Tahoe, El Dorado, CA	T14N R17E Sec. 29
28.	1918 AME	8	Big Meadow, Calaveras, CA	T 7N R17E Sec. 33
29.	1955 DOU	21	Mt. Withington, Socorro, NM	T 4S R 7W Sec. 36
30.	1949 DOU	21	Turkey Flat, Graham, AZ	T 9S R25E Sec. 19
31.	1941 DOU	21	Bear Wallow, pima, AZ	T12S R16E Sec. 5
32.	1938 GIG	14	Hitchcock, pima, AZ	T12S R16E Sec. 22
33.	1964 VAC	17	Pingree-1, Larimer, CO	T 8N R73W Sec. 25
34.	1876 VAC	17	San Antonio, Sandoval, NM	T19N R 3E Sec. 7
35.	1870 VAD	9	Puerto B.A., Durango, Mexico	---- ---- ---- --
36.	1956 PUS	5	Waugochance, Emmet, MI	T39N R 5W Sec. 25
37.	1957 PUS	6	Levering, Emmet, MI	T38N R 4W Sec. 4
38.	1969 PUS	6	Big Falls, Koochiching, MN	T66N R25W Sec. --
39.	1970 PUS	6	Kerrick, pine, MN	T44N R18W Sec. --
40.	1971 PUS	6	Cromwell, Carlton, MN	T49N R20W Sec. --

\* Full specific names of hosts listed here by number are given in Table 3

host trees per population. This number was derived from a formula designed to determine optimal sample sizes when examining the genetics of forest trees (Brown and Moran 1981).

For germination the seeds were soaked in distilled water overnight or until the viscin coat was fully imbibed. The water was then replaced with 2% H<sub>2</sub>O<sub>2</sub> and the seeds placed in a growth chamber set at 14° C with a 12:12 day to night cycle. Germination (extension of the radicle) was used as an indicator of viability of the endosperm (Figure 3C). All species of dwarf mistletoe utilized in this study germinated within 3-4 days under these conditions. Cold storage of certain

species (such as those within the Campylopodum group) for at least four weeks enhanced later seed germination.

To prepare the seeds for electrophoresis, the viscin coat and radicle (if necessary) was dissected away (Figures 3A and 3B) and the endosperm was then stored at -70° C. Owing to their small size (0.5-5.0 mg each), special methods were necessary to extract adequate enzyme samples from individual seeds. An additional disadvantage was that single seeds often provided only enough sample for 2-4 wicks, hence many seeds had to be prepared for a particular run and an individual seed could not be analyzed again. The latter problems were



Table 3.-- Hosts colonized by the dwarf mistletoes used in this study

1.	<u>Abies</u>	<u>concolor</u>
2.	<u>Abies</u>	<u>magnifica</u>
3.	<u>Larix</u>	<u>occidentalis</u>
4.	<u>Picea</u>	<u>engelmannii</u>
5.	<u>Picea</u>	<u>glauca</u>
6.	<u>Picea</u>	<u>mariana</u>
7.	<u>Picea</u>	<u>pungens</u>
8.	<u>Pinus</u>	<u>contorta</u> var. <u>murrayana</u>
9.	<u>Pinus</u>	<u>durangensis</u>
10.	<u>Pinus</u>	<u>edulis</u>
11.	<u>Pinus</u>	<u>flexilis</u>
12.	<u>Pinus</u>	<u>jeffreyi</u>
13.	<u>Pinus</u>	<u>lambertiana</u>
14.	<u>Pinus</u>	<u>leiophylla</u> var. <u>chihuahuana</u>
15.	<u>Pinus</u>	<u>monticola</u>
16.	<u>Pinus</u>	<u>ponderosa</u> var. <u>ponderosa</u>
17.	<u>Pinus</u>	<u>ponderosa</u> var. <u>scopulorum</u>
18.	<u>Pinus</u>	<u>sabiniana</u>
19.	<u>Pinus</u>	<u>strobiformis</u>
20.	<u>Pinus</u>	<u>teocote</u>
21.	<u>Pseudotsuga</u>	<u>menziesii</u>

circumvented by collecting large numbers of seeds and utilizing allele frequency data (not individual genotypes) for the systematic analysis.

For enzyme extraction, a plexiglass plate with 20 cylindrical wells and a steel pestle connected to a motor driven homogenizer was used. The plate was frozen at  $-70^{\circ}\text{C}$  prior to use and kept on ice during grinding. Single seeds were ground in 35-40  $\mu\text{l}$  of a 0.01 M Hepes buffer with 0.025 M 2-mercaptoethanol and 10% (w/v) polyvinylpyrrolidone (PVP-40). The crude extract was absorbed directly onto two Whatman #3 filter paper wicks measuring 4.0 X 9.0 mm. The plate with wicks was then frozen at  $-70^{\circ}\text{C}$  and thawed just before loading the gels.

Enzyme separation was performed using horizontal starch gel (Sigma Co.) electrophoresis as described by Shaw and Prasad (1970). Eight enzyme systems were used in this study. 6-PGD (E.C. # 1.1.1.44), IDH (1.1.1.42), MDH (1.1.1.37), and G-6-PDH (1.1.1.49) were stained from citrate gels modified from Clayton and Treliak (1972). For adequate resolution of 6-PGDH, 10.0 mg of NADP was added to the gel prior to degassing and 5.0 mg to each electrode tray prior to the gel run. ADH (E.C. # 1.1.1.1) and PGI (5.3.1.9) were stained from phosphate gels as described by Selander et al. (1971). PGM (E.C. # 2.7.5.1) and GDH (1.4.1.2) were stained from Tris-Borate gels as described by Ayala et al. (1972). The citrate gels were adjusted to pH 6.5 (gel) and 6.1 (electrode) using N-(3-aminopropyl)-morpholine.

The sample wicks were inserted into a slit in the gel and the gel run until a bromphenol blue marker dye migrated 11.0

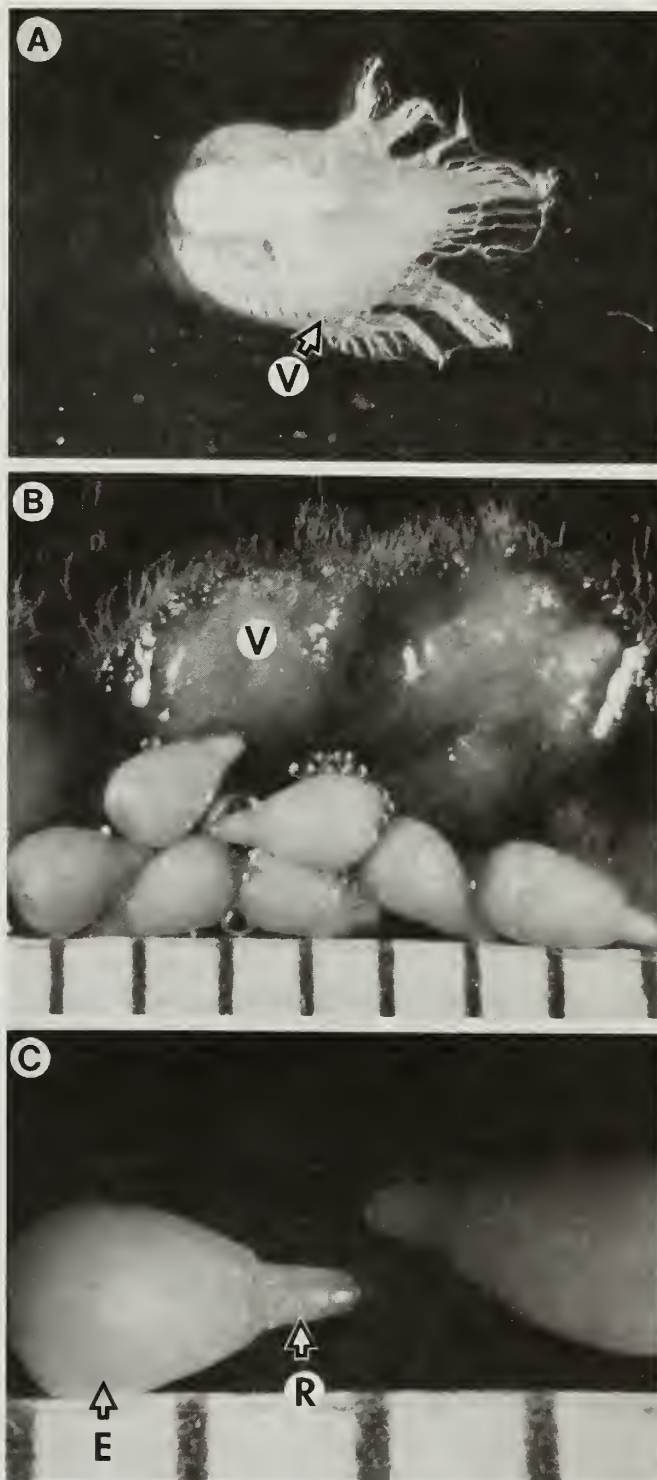


Figure 3a.--Ungerminated seed of Arceuthobium cyanocarpum dried onto a glass slide. 3b. Six day old germinating seedlings of above taxon (in 2%  $\text{H}_2\text{O}_2$ ) with viscin coats removed. 3c. Closeup of seedlings from 3b without viscin coat. Scale in all figures in millimeters. E=endosperm, R=radicle, V= viscin coat.

cm. Enzyme staining for all systems was essentially as reported in Selander et al. (1971), Shaw and Prasad (1970), and Siciliano and Shaw (1976).

Bands for each enzyme locus were assigned relative mobility designations as compared to VAC seeds run as standards on each gel (assigned relative mobility values of "100"). For enzyme systems with two or more loci present on the gel, the most anodal locus was designated number one. Electromorph (= "allele") frequencies were tabulated for each locus and population and these frequencies subjected to analysis using BIOSYS-1, a FORTRAN IV computer program designed to analyze electrophoretically detectable genetic variation (Swofford and Selander 1981).

## RESULTS

Eight enzyme systems representing eleven presumed loci gave interpretable results: 6-PGDH, IDH, MDH-2, MDH-3, G-6-PDH, PGI, ADH-1, ADH-2, PGM-1, PGM-2, and GDH. Allele (electromorph) frequency data were collected since one seed (individual) provided only enough sample for one gel. The mean number of individuals sampled per population for 6-PGDH was 37.4 with a range from 10 to 92. The sample sizes for most of the loci sampled among forty populations of dwarf mistletoes were usually well above ten individuals.

A data matrix of allele frequencies for 11 polymorphic loci for each of the 40 dwarf mistletoe populations is not reproduced here owing to its size (in

Nickrent, 1984). The number of alleles at all loci except G-6-PDH, ADH-2, and GDH exceeded 10. For 6-PGDH and PGI, greater than 20 alleles were detected for the 19 mistletoe taxa studied. For most loci there exists a common electromorph which occurs in high frequencies in many related taxa (e.g. 6-PGDH 150 in the Campylopodium complex). All loci reported here are polymorphic across all taxa, however GDH and G-6-PDH are often monomorphic within one species (the two most conservative systems).

Table 4 gives an estimation of genetic variability per taxon as measured by mean percent polymorphic loci per taxon and the mean number of alleles per locus per taxon. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99. The mean percentage of loci polymorphic across all 19 taxa is 66.6, ranking dwarf mistletoes among the most genetically diverse dicotyledons examined electrophoretically. In comparison to averaged values for angiosperms and gymnosperms (Hamrick et al. 1981), Arceuthobium may be nearly twice as genetically variable as the average dicotyledonous plant. Some of the values in Table 4 should be interpreted with caution since the number of populations sampled for the taxon is small (one for BLU, CAL and others).

Mean levels of heterozygosity per taxon (Nei 1978) are not reported here because the sampled endosperm tissue is triploid, not diploid. The number of expected heterozygote classes is

Table 4.--Genetic variability for 19 taxa of dwarf mistletoe.

<u>Arceuthobium</u> Taxon	Number of Populations	% Polymorphic Loci Per Taxon	Mean Number of Alleles Per Locus Per Taxon
1. <i>A. abietinum</i> f. <i>sp. concoloris</i>	2	50.0	1.90
2. <i>A. abietinum</i> f. <i>sp. magnificae</i>	4	79.5	2.45
3. <i>A. americanum</i>	4	79.5	2.63
4. <i>A. apachecum</i>	4	59.0	2.18
5. <i>A. blumeri</i>	1	45.5	2.00
6. <i>A. californicum</i>	1	90.9	2.70
7. <i>A. campylopodium</i>	3	78.8	2.37
8. <i>A. cyanocarpum</i>	2	63.6	1.80
9. <i>A. divaricatum</i>	1	81.8	2.60
10. <i>A. douglasii</i>	3	72.7	2.70
11. <i>A. gillii</i>	1	54.5	2.50
12. <i>A. laricis</i>	1	63.6	1.70
13. <i>A. microcarpum</i>	2	59.1	2.10
14. <i>A. occidentale</i>	1	81.8	2.20
15. <i>A. pusillum</i>	5	50.9	1.78
16. <i>A. rubrum</i>	1	45.5	1.50
17. <i>A. tsugense</i>	1	72.7	1.90
18. <i>A. vaginatum</i> ssp. <i>cryptopodium</i>	2	63.6	2.60
19. <i>A. vaginatum</i> ssp. <i>durangense</i>	1	72.7	2.80
	Mean	66.6	Mean 2.23



necessarily elevated with increasing ploidy level thereby precluding the value of direct comparisons with diploids. The observed banding patterns for the triploid mistletoe endosperms conformed very well to expected patterns. Enzyme staining intensity and the number of bands present on a gel allowed accurate genotype assessments for most homozygote and heterozygote patterns seen. Interpretations of triploid enzyme banding patterns for monomeric and dimeric isozymes will be reported separately (Nickrent 1984).

phenograms were generated using the unweighted pair group method with arithmetic averaging (UPGMA) as described in Sneath and Sokal (1973). The genetic similarity measure reported here is that of Nei (1978) shown in Figure 4. Other similarity measures such as by Rogers (1972) resulted in similar phenograms but differed in the percent standard deviation (Fitch and Margoliash 1967). The standard deviations for the above two measures were 56.717 and 22.454, respectively. The two measures differed primarily in the branching array for taxa within the

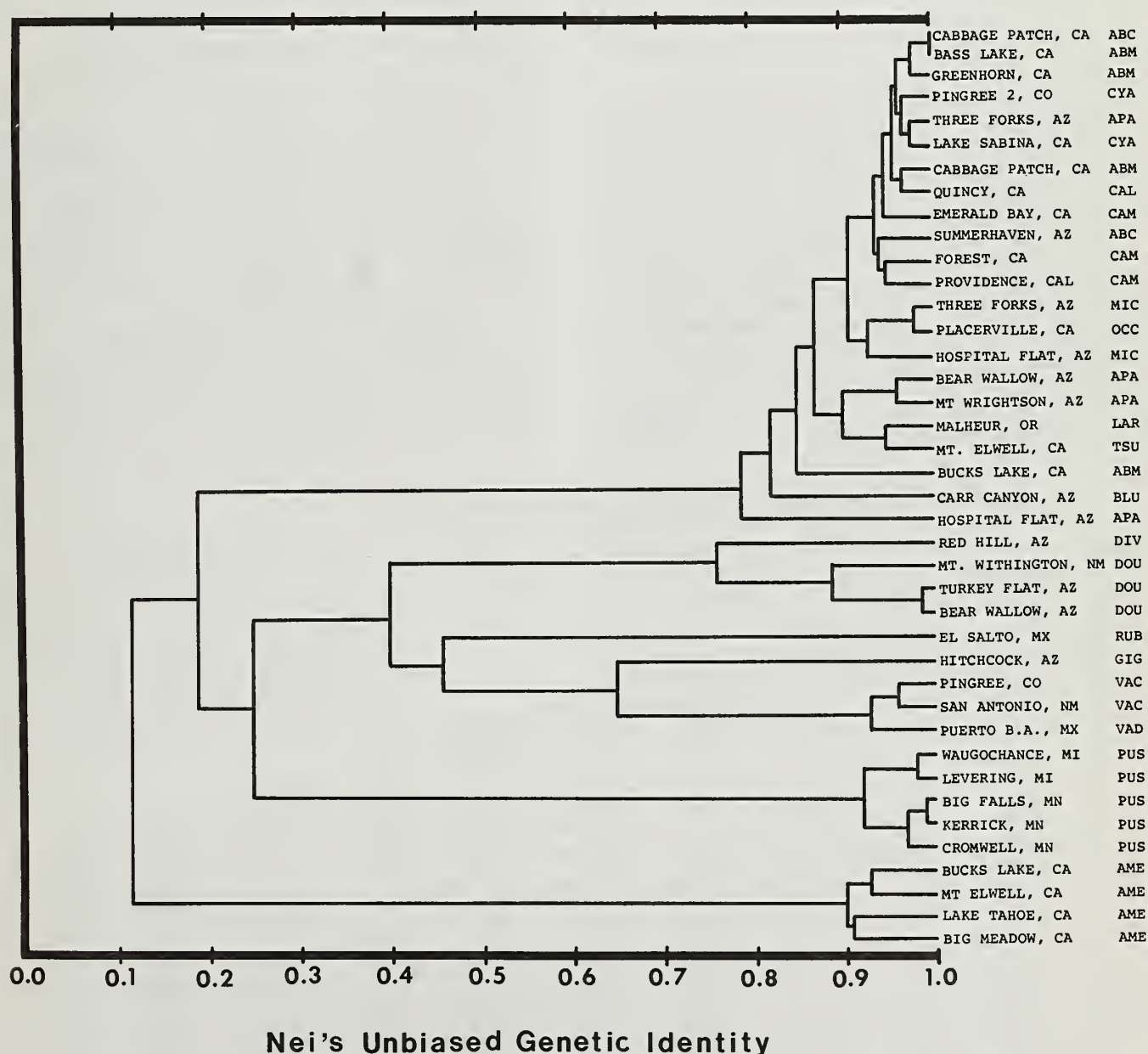


Figure 4.--UPGMA phenogram for the 40 dwarf mistletoe populations listed in Table 2 utilizing Nei's (1978) unbiased coefficient of genetic identity. See Table 1 for full taxonomic names here given three letter abbreviations.

Campylopodum complex. Also, Nei's (1978) measure showed a higher percent similarity for all taxa included in the analysis.

A matrix of genetic distance values (Nei 1978), averaged by species, is shown in Table 5. (mean distance values printed above, ranges below). The matrix for interpopulational genetic distances is not included owing to its size. Taxa were arranged according to the subgeneric, sectional, and series hierarchy outlined in Hawksworth and Wiens (1972) which is reproduced for the taxa under consideration here in Table 1. Ranges of the distance coefficients were used to examine the integrity of this arrangement by section (Table 6). An alternate sectional and series arrangement, based on the isozyme phenogram (Figure 4) is shown in Table 7. The means and ranges of the distance values, given this hierarchal arrangement, are shown in Tables 8 and 9.

The terms "section" and "series" are used in this paper for groupings as visualized from the isozyme results and carry no special taxonomic significance. The term "phenon" (Sneath and Sokal 1973) could be substituted for these terms. The categories were arbitrarily determined by examining the groupings of Figures 4 and then making subjective judgements as to the most natural subgeneric arrangement. The names given these categories are, in some cases, identical to those proposed by Hawksworth and Wiens (1972) even though the taxa included are not identical. Names such as "Series Divaricata" are proposed

only to accomodate the altered arrangement based on isozyme evidence.

When examining the sectional integrity of the arrangement shown in Table 6, the most heterogeneous group is Section Minuta with a mean distance value of 0.674 and ranges from 0.010-1.327. This contrasts with the mean intrasectional values for Section Americana (0.101) and vaginata (0.248). The highest value (2.372) for Section Campylopoda results from the inclusion of two taxa (DIV and RUB) which show greater affinity to other sections.

Tables 8 and 9 give the distance values for a sectional and series arrangement based on the isozyme phenograms. This arrangement reduces the variation in Section Campylopoda from a mean of 0.360 to 0.122. With this system of ranking, Section vaginata is the most heterogeneous group (mean of 0.690), owing mainly to the inclusion of RUB. This is illustrated by examining the intragroup integrity at the series level. Here, RUB is excluded from the remainder of the section and the distances for Series divaricata and vaginata fall to 0.18 and 0.24 respectively. When all six series are examined, none of the intragroup values exceeds a distance 0.25.

A number of similarities and discrepancies appear when the phenetic analysis of Hawksworth and Wiens (1972) is compared to the present isozyme analysis. First, both methods segregate AME quite early from the remainder of the genus.

Table 5.--Interspecific genetic distance values (Nei 1978). Mean values above, ranges below.

	1	2	3	4	5	6	7	8	9	10
1. AME	0.101 (0.070-0.127)									
2. GIG	1.463 (1.236-1.705)	***** (*****-*****)								
3. VAD	1.683 (1.470-2.049)	0.336 (0.336-0.336)	***** (*****-*****)							
4. VAC	1.751 (1.475-2.124)	0.489 (0.448-0.529)	0.069 (0.056-0.082)	0.037 (0.037-0.037)						
5. OCC	2.670 (2.120-3.274)	1.449 (1.449-1.449)	1.114 (1.114-1.114)	1.201 (1.145-1.258)	***** (*****-*****)					
6. CYA	2.636 (2.184-3.116)	1.512 (1.459-1.565)	1.105 (1.089-1.121)	1.184 (1.125-1.227)	0.063 (0.057-0.070)	0.034 (0.034-0.034)				
7. BLU	2.944 (2.622-3.384)	1.641 (1.641-1.641)	0.974 (0.974-0.974)	1.008 (0.990-1.026)	0.196 (0.196-0.196)	0.173 (0.173-0.173)	***** (*****-*****)			
8. APA	2.526 (1.814-3.462)	1.356 (1.098-1.464)	1.039 (0.832-1.208)	1.127 (0.850-1.368)	0.181 (0.050-0.343)	0.110 (0.022-0.233)	0.281 (0.155-0.418)	0.130 (0.043-0.238)		
9. CAM	2.529 (2.064-3.215)	1.430 (1.427-1.433)	1.107 (1.028-1.148)	1.200 (1.061-1.298)	0.086 (0.069-0.101)	0.062 (0.029-0.104)	0.188 (0.169-0.200)	0.148 (0.043-0.309)	0.063 (0.050-0.082)	
10. TSU	3.063 (2.689-3.611)	1.588 (1.588-1.588)	1.150 (1.150-1.150)	1.215 (1.191-1.238)	0.203 (0.203-0.203)	0.136 (0.115-0.156)	0.285 (0.285-0.285)	0.119 (0.061-0.185)	0.192 (0.125-0.250)	***** (*****-*****)
11. CAL	2.565 (2.168-3.078)	1.397 (1.397-1.397)	1.038 (1.038-1.038)	1.119 (1.073-1.165)	0.030 (0.030-0.030)	0.044 (0.042-0.046)	0.121 (0.121-0.121)	0.135 (0.032-0.269)	0.059 (0.049-0.065)	0.150 (0.150-0.150)
12. DIV	1.463 (1.344-1.576)	1.007 (1.007-1.007)	0.580 (0.580-0.580)	0.597 (0.592-0.603)	1.995 (1.995-1.995)	2.019 (1.908-2.130)	1.535 (1.535-1.535)	1.947 (1.868-2.004)	1.898 (1.843-1.975)	2.372 (2.372-2.372)
13. LAR	2.756 (2.353-3.407)	1.474 (1.474-1.474)	1.081 (1.081-1.081)	1.161 (1.115-1.208)	0.130 (0.130-0.130)	0.142 (0.110-0.174)	0.259 (0.259-0.259)	0.174 (0.140-0.251)	0.166 (0.123-0.224)	0.052 (0.052-0.052)
14. MIC	2.525 (2.049-3.447)	1.395 (1.340-1.449)	1.071 (1.064-1.077)	1.148 (1.083-1.212)	0.039 (0.019-0.059)	0.089 (0.070-0.106)	0.252 (0.230-0.274)	0.201 (0.058-0.408)	0.140 (0.099-0.192)	0.202 (0.125-0.278)
15. ABC	2.637 (2.234-3.127)	1.491 (1.446-1.537)	1.077 (1.043-1.111)	1.158 (1.080-1.233)	0.080 (0.077-0.083)	0.044 (0.020-0.061)	0.118 (0.111-0.125)	0.133 (0.027-0.277)	0.067 (0.040-0.105)	0.184 (0.136-0.232)
16. ABM	2.557 (2.111-3.160)	1.437 (1.408-1.475)	1.055 (1.024-1.104)	1.150 (1.062-1.288)	0.094 (0.072-0.148)	0.066 (0.019-0.166)	0.186 (0.139-0.273)	0.131 (0.035-0.339)	0.083 (0.040-0.171)	0.135 (0.063-0.216)
17. RUB	2.562 (2.330-2.968)	0.784 (0.784-0.784)	0.733 (0.733-0.733)	0.777 (0.753-0.800)	1.229 (1.229-1.229)	1.248 (1.244-1.253)	1.043 (1.043-1.043)	1.257 (1.121-1.433)	1.255 (1.122-1.359)	1.231 (1.231-1.231)
18. DOU	1.465 (1.292-1.732)	1.166 (1.127-1.194)	0.816 (0.721-0.874)	0.826 (0.668-0.913)	2.375 (2.216-2.638)	2.164 (1.970-2.566)	1.505 (1.412-1.671)	2.229 (1.985-2.693)	2.096 (1.688-2.554)	2.331 (2.065-2.793)
19. PUS	1.920 (1.528-2.255)	1.552 (1.438-1.666)	1.354 (1.258-1.469)	1.423 (1.290-1.638)	1.912 (1.687-2.182)	1.952 (1.566-2.379)	2.105 (1.734-2.459)	1.766 (1.454-2.037)	1.825 (1.462-2.191)	2.240 (2.048-2.436)



Table 6.--Distance coefficients (Nei 1978) averaged by section\*

Section	# Pops	1	2	3	4
1. Americana	4	0.101 (0.070-0.127)			
2. Vaginaturn	4	1.662 (1.236-2.124)	0.248 (0.037-0.529)		
3. Campylopoda	24	2.564 (1.344-3.611)	1.167 (0.580-1.641)	0.360 (0.005-2.372)	
4. Minuta	8	1.750 (1.292-2.255)	1.239 (0.668-1.666)	1.946 (0.218-2.850)	0.674 (0.010-1.327)

\*Sectional arrangement according to Hawksworth and Wiens (1972)

Also, both studies suggest that the Vaginata group exhibits more variation, both morphological and genetic, than Section Campylopoda. The isozyme analysis does not, however, support the placement of DOU and PUS together in one Section (Minuta). Also, the pinyon dwarf mistletoe, DIV, segregates most closely with DOU, not the Campylopoda complex as suggested by the morphometric analysis. Arceuthobium rubrum (RUB), placed within its own Series of Section Campylopoda by Hawksworth and Wiens (1972) clusters with a group of three taxa generally representing Section Vaginata. It is of interest that the morphometric analysis (Figure 1) shows this taxon (with A. strictum, which was not included in this study), having greater affinity with Section Vaginata than any other members of Section Campylopoda.

The second major dichotomy of the genus based on isozyme evidence segregates those members placed in Section Campylopoda, Series Campylopoda by Hawksworth and Wiens (1972). The clustering patterns for those dwarf mistletoes included within Series Campylopoda do not support segregation of separate species. Ten taxa (ABC, ABM,

APA, CAL, CAM, CYA, LAR, MIC, OCC, and TSU) included in this group share a high degree of genetic similarity (greater than 80%). Six of these ten taxa (ABC, ABM, APA, CAM, CYA, and MIC) are represented in this study by more than one population, however the cluster analysis does not unambiguously group them together. Some tendency to group according to species, as defined by Hawksworth and Wiens (1972), is seen in some populations of Arceuthobium abietinum s. l., CAM, and APA.

These discrepancies in grouping the taxa considered species within the Campylopoda complex stand in sharp contrast to the well resolved species segregating in the lower half of the phenograms shown in Figure 4. Arceuthobium vaginatum s. l., AME, DIV, DOU, GIG, PUS, and RUB are formed at specific identity values of ca. 75%. The two subspecies of A. vaginatum, VAC and VAD, segregate at ca. 92% similarity using Nei's (1978) index. Many of the populations considered separate species according to Hawksworth and Wiens (1972) show equally high (75% or greater) levels of identity.

Table 5.--Interspecific genetic distance values (Nei 1978). Mean values above, ranges below

	11	12	13	14	15	16	17	18	19
11. CAL	***** (*****-*****)								
12. DIV	1.886 (1.886-1.886)	***** (*****-*****)							
13. LAR	0.112 (0.112-0.112)	2.122 (2.122-2.122)	***** (*****-*****)						
14. MIC	0.075 (0.061-0.089)	2.030 (1.987-2.074)	0.147 (0.094-0.199)	0.079 (0.079-0.079)					
15. ABC	0.030 (0.020-0.040)	1.917 (1.898-1.936)	0.177 (0.140-0.213)	0.124 (0.108-0.155)	0.037 (0.037-0.037)				
16. ABM	0.054 (0.026-0.116)	1.967 (1.950-2.005)	0.122 (0.048-0.170)	0.140 (0.083-0.241)	0.074 (0.005-0.208)	0.077 (0.017-0.131)			
17. RUB	1.199 (1.199-1.199)	1.423 (1.423-1.423)	1.160 (1.160-1.160)	1.090 (1.008-1.172)	1.230 (1.194-1.266)	1.209 (1.154-1.273)	***** (*****-*****)		
18. DOU	2.146 (1.983-2.468)	0.279 (0.218-0.324)	2.409 (2.181-2.850)	2.546 (2.352-2.744)	2.111 (1.921-2.466)	2.171 (1.890-2.711)	1.408 (1.392-1.436)	0.080 (0.010-0.120)	
19. PUS	1.808 (1.548-2.083)	1.156 (1.053-1.239)	2.027 (1.857-2.256)	1.912 (1.606-2.206)	1.710 (1.351-2.096)	1.901 (1.601-2.225)	2.194 (1.892-2.463)	1.204 (1.113-1.327)	0.057 (0.014-0.121)



Table 7.--Subgeneric classification of New World dwarf mistletoes used in this study based on isozyme evidence.

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A. Section <u>Arceuthobium</u> *
<u>A. americanum</u>
B. Section <u>Pusilla</u>
<u>A. pusillum</u>
C. Section <u>Campylopoda</u>
<u>A. abietinum</u> f. sp. <u>concoloris</u>
<u>A. abietinum</u> f. sp. <u>magnificae</u>
<u>A. apachecum</u>
<u>A. blumeri</u>
<u>A. californicum</u>
<u>A. campylopodum</u>
<u>A. cyanocarpum</u>
<u>A. laricis</u>
<u>A. microcarpum</u>
<u>A. occidentale</u>
<u>A. tsugense</u>
D. Section <u>Vaginata</u>
1. Series <u>Vaginata</u>
<u>A. gillii</u>
<u>A. vaginatum</u> ssp. <u>cryptopodum</u>
<u>A. vaginatum</u> ssp. <u>durangense</u>
2. Series <u>Rubra</u>
<u>A. rubrum</u>
3. Series <u>Divaricata</u>
<u>A. divaricatum</u>
<u>A. douglasii</u>

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\* The categories "Section" and "Series" and their names carry no special taxonomic meaning in this study (see text).

#### DISCUSSION

The above evidence indicates that the two classifications, one based on morphology, physiology, and host information (Hawsworth and Wiens 1972), and the present isozyme analysis, provide different interpretations of relationships in the genus. This is not unusual for it has been well established that taxonomic data derived from disparate sources can often lead to entirely different classifications (Mayr 1981; Patton and Avise 1983).

Three major difficulties of biological classification are that 1) we are constrained by the evidence at hand (i.e. extant taxa and fossils) and common ancestry must therefore be presumed, 2) evolutionary change over time may occur at very different rates for different taxa, and 3) we may or may not be successful in attempting to use any one system for both information storage and retrieval and as a basis for biological generalizations and explanations (Lehman 1971; Kavanaugh 1977; Mayr 1981; and Jensen 1983). At least three major schools of thought currently attempt to unambiguously determine the degree of relatedness between taxa, the phenetic, cladistic, and evolutionary taxonomic methods (Mayr 1981). Although there are strong proponents for both the purely phenetic and purely cladistic schools, biologists working with a variety of groups, each with its own evolutionary peculiarities, seem to be striving toward a consensus where information from each method can be incorporated to provide a suitable, workable evolutionary classification. This position has been advanced by Grant (1982) for plants and Mayr (1969; 1981) for animals. Mayr (1981) states that the goal of the evolutionary taxonomist is to "...construct classifications that reflect both of the two major evolutionary processes, branching and divergence (cladogenesis and anagenesis)".

Two examples of apparent morphological convergence involve DIV compared with the Campylopodum complex and DOU compared with PUS. The pinyon pine dwarf mistletoe interestingly shows affinity with DOU, the principle parasite of Douglas fir. Pinyon pines are generally lower elevation species in the western U.S., hence the degree of sympatry between DIV and most other dwarf mistletoe taxa is low. The placement of DIV nearer to DOU than to any taxon in the Campylopodum complex is a radical departure from the treatment in Hawsworth and Wiens (1972).

Table 8.--Distance coefficients (Nei 1978) averaged by section\*

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Section	# Pops	1	2	3	4
1. Americana	4	0.101 (0.070-0.127)			
2. Pusilla	5	1.920 (1.528-2.255)	0.057 (0.014-0.121)		
3. Campylopoda	22	2.614 (1.814-3.611)	1.881 (1.351-2.459)	0.122 (0.005-0.418)	
4. Vaginata	9	1.675 (1.236-2.968)	1.413 (1.053-2.463)	1.621 (0.832-2.850)	0.694 (0.010-1.436)

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\*Sectional arrangement according to isozyme evidence.

Table 9.--Distance coefficients (Nei 1978) averaged by series\*

Series	# Pops	1	2	3	4	5	6
1. Americana	4	0.101 (0.070-0.127)					
2. Pusilla	5	1.920 (1.528-2.255)	0.057 (0.014-0.121)				
3. Campylopoda	22	2.614 (1.814-3.611)	1.881 (1.351-2.459)	0.122 (0.005-0.418)			
4. Vaginata	4	1.662 (1.236-2.124)	1.438 (1.258-1.666)	1.206 (0.832-1.641)	0.248 (0.037-0.529)		
5. Divaricata	4	1.465 (1.292-1.732)	1.192 (1.053-1.327)	2.137 (1.412-2.850)	0.855 (0.580-1.194)	0.180 (0.010-0.324)	
6. Rubra	1	2.562 (2.330-2.968)	2.194 (1.892-2.463)	1.210 (1.008-1.433)	0.768 (0.733-0.800)	1.412 (1.392-1.436)	***** (*****-*****)

\*Series arrangement according to isozyme evidence.

\*\*\*\*\* Only one population included.

It could be stated that biochemical divergence occurred in DIV (from the *Campylopodum* group) and that similarity to DOU is coincidental, i.e. the bands which migrate to the same position on a gel are "electromorphs" and do not represent homologous proteins. Electromorphs appearing the same among different taxa may, under altered electrophoretic conditions, prove to be different (Coyne et al. 1979). In this case, however, when the total number of shared electromorphs is examined, a more reasonable explanation is that similarity is by descent. Both DIV and DOU are fixed for GDH-66 which is absent in all other taxa. In addition, they share: 6-PGD-65, 6-PGD-100, PGI-154, PGI-178, ADH-1-100, ADH-1-132, ADH-1-164, ADH-2-100, PGM-1-76, PGM-1-97, PGM-2-97, PGM-2-100, G-6PDH-100, MDH-2-76, MDH-2-100, and MDH-3-205. The probability of independently evolving this complement of electromorphs is very remote.

The phylogenetic relationships between AME, PUS, and DOU may not be as distant as is suggested by Figure 2 from Hawksworth and Wiens (1972). In this figure it is surprising to see Section *Minuta* as being the most recently derived component of all the members of Subgenus *vaginata*, especially since these taxa do show association on the morphometric dendrogram (Figure 1).

Despite the early segregation of AME from all other taxa utilized in this isozyme study (supporting its retention in a separate subgenus), the affinities of this species to Section *Minuta* (specifically PUS) still remains in question. These three taxa (AME, DOU, PUS) share the following features:

- 1) spring flowering
- 2) an asynchrony of male and female meiosis
- 3) primarily isophasic broom formation
- 4) extensive geographic ranges
- 5) high tendencies to "cross-over" (i.e. colonize secondary host species)
- 6) specific fungal hyperparasite colonization (*Wallrothiella arceuthobii*; Kuijt 1963).
- 7) highest mean number of electromorphs (28 for AME, 29 for DOU compared to a mean of 23.6 across 11 loci for all 19 taxa used in this isozyme analysis).

Given the criterion of secondary branching type (i.e. verticillate vs. flabellate), two subgeneric categories could be established irrespective of any other characters. Kuijt (1970), however, pointed out that strict adherence to the type of secondary branching pattern when delimiting subgenera is not suitable, especially with Old World members such as *Arceuthobium minutissimum*. The latter species is placed in Subgenus *Arceuthobium* by Kuijt (1970) and Hawksworth and Wiens (1972) even in the absence of evidence on secondary branching (owing to the extreme morphological reduction). A similar situation may well exist for the eastern dwarf mistletoe, PUS, where only primary branching has been reported. In addition, the frequency of verticillate or flabellate branching can be a variable



character. Mark and Hawksworth (1981) noted that CAM may show 0-18% verticillate branching and OCC 0-10%. This variable condition was also noted in VAC and CYA. The degree of verticillate secondary branching in various members of subgenus Arceuthobium can vary tremendously between taxa. This character was used by Hawksworth and Wiens (1976) to segregate A. juniperi-procerae (native to Africa) from A. oxycedri (wide ranging in Europe and Asia). The former taxon displays less than 3% verticillate secondary branching whereas the latter shows ca. 45% whorled arrangement.

The phenetic analysis based on isozyme evidence segregates four major groups at the 0.3% similarity level (Figure 4). This arrangement by section and series, based upon the isozyme phenograms, provides a more equitable hierarchy, given electrophoretic distance values, than the arrangement according to Hawksworth and Wiens (1972). The affinities of RUB with Section vaginata is shown with both the isozyme analysis (Figure 4) and the morphological analysis (Figure 1), although more strongly with the former measure. The most variable section within this arrangement is Vaginata. This agrees with the results of the morphometric analysis of Hawksworth and Wiens (1972).

Defining a species in the Campylopodum complex is difficult because of the overall high degree of genetic similarity. Since species limits are well defined in some cases (AME, DOU, PUS, etc.) but not in others, it is proposed that either 1) the complex does not contain distinct species, or 2) the complex is composed of closely related (sibling) species, possibly undergoing more rapid (and more recent) radiation than other members of the genus. The evidence presented here does not discount the scheme of relationships within the Campylopodum complex as proposed by Kuijt (1955) (Table 1). As has long been recognized, host relationships are crucial to developing an understanding of speciation in this group. When commenting that evolutionary divergence is independent of reproductive isolation, Levin (1979) said species are "the total assemblage of adaptive peaks with continuity between the peaks." For the Campylopodum complex, these peaks may well correspond to the major host species.

It would be desirable, then, to utilize all of the information at hand in order to construct a model phylogeny for this interesting genus of parasites. Whether the methodology involves cladistic or phenetic techniques, the most rational approach to dwarf mistletoe classification would be an attempt to use the information from each area in the process of synthesizing a phylogeny. The following conclusions can be derived from the results of this study:

1) Arceuthobium shows very high levels of genetic variability.

2) AME represents the most primitive New World member of the genus included in this study.

3) DOU and PUS are not recent derivatives from the Campylopodum stock. The phylogenetic affinities of the former species are with Section Vaginata.

4) DOU and PUS, as shown by isozyme evidence, are not closely related to one another.

5) DIV appears to reside more naturally with Section vaginata than with Section Campylopada.

6) The affinities of VAC, VAD, and GIG are supported by the previous analysis of Hawksworth and Wiens (1972) and the present isozyme study. This group is both morphologically and genetically more variable than other members of the genus.

7) Eleven taxa within the Campylopodum complex show high levels of genetic similarity (ca. 80%). These are: ABC, ABM, APA, BLU, CAL, CAM, CYA, LAR, MIC, OCC, and TSU. In comparison to species limits of AME, DOU, and PUS, these taxa show greater inter-taxon similarity.

8) The Mexican taxon, RUB, appears to occupy a transitional position between the Campylopodum and vaginatum groups.

The placement of DIV either with Section vaginata or Campylopada remains the major discrepancy between the former and present proposed classifications. This impasse can only be resolved by broadening the scope of genetic investigation to include more Mexican and Central American taxa such as Arceuthobium pendens. Other Mexican taxa central to developing an understanding of phylogenetic relationships in this genus include: A. abietis-religiosae, A. verticilliflorum, A. gillii ssp. nigrum, A. vaginatum ssp. vaginatum, A. strictum, and A. globosum. The inclusion of Old World taxa would also be extremely helpful, especially to establish the proper placement of such taxa as A. pusillum at the subgeneric level.

Questions raised by this isozyme study involving the evolution and taxonomy of certain component taxa include:

1) What is the evolutionary and biogeographic relationship between DIV and DOU?

2) What is the phylogenetic status of PUS and its relationship to AME,



especially in reference to subgeneric concepts in the genus?

3) What is the specific status of component taxa in the closely related Campylopodium complex? Are these taxa very recently evolved (or evolving) species segregating along host lines? If so, what are the selective forces driving this radiation?

4) Given high levels of genetic variability, the evolutionary potential of these parasites is quite great. How must this influence our concepts of speciation and pathogenicity in Arceuthobium with respect to effective control?

5) How must the current classification system be modified to incorporate this additional information on genetic relationships?

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# Genetic Variability in the Dwarf Mistletoes *Arceuthobium vaginatum* subsp. *cryptopodum* and *A. americanum* on their Primary and Secondary Hosts<sup>1</sup>

Yan B. Linhart<sup>2</sup>

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Abstract.--Electrophoretic analyses of genetic variation have been carried out on *Arceuthobium americanum* and *A. vaginatum* subsp. *cryptopodum*. Several polymorphic loci have been detected in both. A comparison of *A.v. cryptopodum* populations growing on *Pinus ponderosa*, its primary host, and *P. contorta*, a secondary host, indicated significant heterogeneity of allele frequencies between the two populations at two of the three loci tested. There was no evidence of differentiation at one locus in *A. americanum* whose primary host is *P. contorta* and secondary host is *P. ponderosa*.

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## INTRODUCTION

Dwarf mistletoes of the genus *Arceuthobium* are common parasites on pines and other conifers of the Rocky Mountains. Each of the 3 main pine species in northern Colorado is the primary host of an *Arceuthobium* species. *Pinus ponderosa* is parasitized by *A. vaginatum* subsp. *cryptopodum*, *P. contorta* is parasitized by *A. americanum* and *P. flexilis* is parasitized by *A. cyanocarpum*. However, all three of these *Arceuthobium* species also grow on occasion on the "wrong" two pine species which then can serve as secondary hosts. The frequency with which this happens is variable but all nine possibilities have been seen in the field (Hawksworth and Peterson 1959, Hawksworth et al. 1975, Hawksworth 1978).

As this symposium attests, a great deal is known about the ecology, systematics and destructive features of *Arceuthobium*. The extent of its variability and other features of its genetics are less well known and are in need of study for practical and theoretical reasons. The practical reasons are based on the fact that it is the most damaging disease of conifers in many regions in western North America and Canada (Hawksworth 1978, 1983). Methods of reducing damage levels depend on proper biological management including thinning and pruning

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(Scharpf and Parmeter 1978) and can benefit from a better understanding of the biology of the genus. The theoretical reasons include the fact that little is known about the evolutionary genetics of parasitic angiosperms (Kuijt 1969, Calder and Bernhardt 1983) and how they compare to other angiosperms in terms of their levels of variability (Hamrick et al. 1979), extent of geographic variation and population differentiation (Stebbins 1950, Endler 1977), and the possibilities of co-evolution with their hosts (Futuyma and Slatkin 1983).

The primary objective of this paper is to discuss preliminary analyses of electrophoretically-detectable genetic variation in *A.v.* subsp. *cryptopodum* and *A. americanum*. Two questions are asked:

1) Which loci are variable in which species and under which electrophoretic conditions. This will be useful in determining methodology of future studies.

2) Are there any differences between the genetic constitutions of a given *Arceuthobium* species on its primary host and on a secondary host? This question is useful in the context of determining whether there is a possibility of host race formation. This is observed in many insects which can feed upon a variety of plant hosts and show significant genetic differentiation as a result.

## MATERIALS AND METHODS

Shoot samples of *A. vaginatum* subsp. *cryptopodum* (henceforth *A.v.*) and *A. americanum* (henceforth *A.a.*) were collected on *Pinus*

ponderosa (P.p.) and Pinus contorta (P.c.). In addition A.v. were collected on P. flexilis (P.f.). All collecting was done in a mixed stand containing all three species in an area north of Nederland, Colorado, U.S.A. (Lat. 39° 57'N., Long. 105° 35'W.) at an elevation of 2600 m. Collecting was not done randomly. Efforts were made to collect colonies from a variety of locations on a given tree. Also, colonies were collected from a variety of trees. Three to six colonies were collected from most single trees, with a maximum of 16 A.v. from one P.c. because P.c. with A.v. infestations were difficult to find. Both species were in flower, and the sex could be determined for all colonies of A.a. and many colonies of A.v.

The shoot samples were brought back to the laboratory and, within one day, ground in liquid N and then mixed with a buffer, following methods described in Mitton et al. (1979). The system in which the samples were run include the discontinuous buffer described in Mitton et al. (1977) and the continuous buffer described in King and Dancik (1983).

Allele frequencies were compared between populations growing on either host; they were also compared between sexes. Sample sizes used to determine allele and genotype frequencies for a given category, i.e. host population or sex, are 39 to 47. Differences were tested with the chi-square test of Workman and Niswander (1970).

## RESULTS AND DISCUSSION

### Methodology

For any given protein, the same buffer system gave proper resolution for material from all species of mistletoe sampled (i.e. A.a., A.v. and a few A. cyanocarpum collected from P.f.).

The buffer we have used most commonly for ponderosa pine tissue and described in Mitton et al. (1977) was especially useful for the following enzymes. The list also indicates magnitude of genetic variability as H = High, meaning there are at least two alleles with the most common allele at frequency  $P < 0.90$  or L = Low with  $P > 0.95$ . Alcohol Dehydrogenase (A.D.H.) two loci, the faster one is H, the slower one is L. Glutamate Dehydrogenase (G.D.H.) L; Phosphoglucose Isomerase (P.G.I.) H; Peroxidase (PER) several loci, one H others L.

The continuous buffer of King and Dancik (1983) was especially good for the following enzymes. Aconitase (ACO) L; Isocitrate Dehydrogenase (I.D.H.) L; 6-Phosphogluconate Dehydrogenase (6-P.G.D.H.) L; Malate Dehydrogenase (M.D.H.) H; Phosphoglucumutase (P.G.M.) 2 loci, both H.

Results are reported here on only a few of these loci. In all cases the loci are presumptive because no control-pollinations were done and no comparisons were done between parents and progenies. However, they are considered to be

loci based on extensive previous experience with these enzymes in many plant species both in our laboratory and elsewhere (Hamrick et al. 1979). Appropriate controls were run with fresh versus older, refrigerated tissues, and with several samples of varying developmental sizes from one colony. Furthermore all loci showed distributions of genotypes which were very close to those expected under Hardy-Weinberg equilibrium.

### Variation in A. americanum

A. americanum was abundant on P. contorta in the stand sampled. Most trees had at least a few colonies. A. americanum also colonized P. ponderosa readily. Because an effort was made to collect balanced samples of both ♀ and ♂, the sex ratio cannot be determined from these samples, but both sexes were common on both pines. Patterns of genetic variation in A.a. are summarized in Table 1. There are no significant differences in allele frequencies between sexes or between A.a. on the two hosts. The lack of differentiation in allele frequencies parallels the lack of differentiation at the morphological level.<sup>3</sup> Observed genotypic frequencies do not differ significantly from those expected under Hardy-Weinberg equilibrium for either sex or for populations collected from the two hosts.

Table 1.--Frequency of two most common alleles at the PGI locus in A. americanum growing on either of two hosts (C = P. contorta; P = P. ponderosa) or of either sex. Chi square tests the difference between hosts or sexes, and P indicates significance level.

Locus and alleles	Host		Sex	
	C	P	♀	♂
P.G.I. Frequency				
Allele 1	0.13	0.10	0.05	0.17
Allele 3	0.81	0.78	0.83	0.76
$\chi^2$ (P)	2.63(P<0.20)		1.23(P>0.20)	

### Variation in A. vaginatum

A. vaginatum is common on P. ponderosa in the stand sampled, but, in contrast to the frequent occurrence of A.a. on the "wrong" host, A.v. is not very common on P. contorta in this area. Few P.c. have A.v. and when they do have it, they support few colonies. A.v. was also found on one P. flexilis. As in A.a., both sexes were common on both pines.

<sup>3</sup>Hawksworth, Frank G. 1984. Personal conversation. USDA Forest Service, Rocky Mountain Forest Experiment Station, Ft. Collins, Colo.



There are significant differences in allele frequencies at the P.G.I. and A.D.H. loci between A.v. on P.p. versus P.c. (Table 2). This differentiation parallels the differentiation in morphology and color between A.v. on P.p. versus P.c. as noted by Hawksworth.<sup>3</sup>

Table 2.--Frequency of most common allele(s) at three loci in *A. vaginatum* subsp. *cryptopodum* growing on either of two hosts or of either sex. Chi square tests the difference between hosts or sexes, and P indicates significance level.

Locus and allele(s)	Host		Sex	
	C	P	♀	♂
P.G.I. Frequency of most common alleles				
Allele 2	0.26	0.10	0.20	0.15
Allele 4	0.33	0.40	0.39	0.32
Allele 5	0.20	0.29	0.25	0.38
X <sup>2</sup> (P)	14.37 (P<0.01)		1.10 (P>0.50)	
A.D.H. Frequency				
Allele 2	0.78	0.94	0.69	0.82
X <sup>2</sup> (P)	8.60 (P<0.005)		0.36 (P>0.50)	
PER Frequency				
Allele 2	0.65	0.63	0.52	0.67
X <sup>2</sup> (P)	0.36 (P<0.50)		3.71 (P=0.06)	

There are no statistically significant differences in allele frequencies between sexes of A.v. at two loci, though values are close to significance at the PER locus.

Observed genotypic frequencies do not differ significantly from those expected under assumptions of Hardy-Weinberg equilibrium for any locus, either sex or either host population.

The sample of A.v. from a single P.f. host consisted of five colonies only. The plants did not differ strikingly in either morphological or electrophoretic phenotypes from the other host populations.

#### GENERAL DISCUSSION

The *Arceuthobium* species tested so far are quite variable genetically in that more than one third of the loci tested to date are polymorphic. In very detailed analyses, Nickrent (this volume) concludes that *Arceuthobium* as a genus is markedly more variable than most plant species reported on to-date (Hamrick et al. 1979). This variability contrasts with the intra-specific uniformity in flavonoid chemistry (Crawford and Hawksworth, 1979) and morphology in this genus. At the P.G.I. locus, local variability is especially remarkable: there are 5 alleles in A.a. and 8 alleles in A.v. This locus is known to be highly variable in other plants (Gottlieb 1981). Nevertheless, this variability is unusual

considering that the total area sampled for A.v. is no more than 10 km<sup>2</sup> and for A.a. it is about 2 ha. In A.v. two locations have been sampled, and two of the alleles detected at one location in a small sample of 30 individuals have not been detected at the other (Linhart, unpublished data). The difference may be the result of limited sampling, but it does suggest that there is a good deal of genetic heterogeneity between populations. This is in keeping with the generally patchy distribution of *Arceuthobium* even within homogeneous pine forests (Hawksworth, 1978).

The genetic constitution of A.a. populations at one locus is very similar regardless of hosts (Table 1). This is not true for A.v. where there is significant difference between the population on P.p. and P.c. both at the electrophoretic (Table 2) and morphological<sup>3</sup> levels. Considerably more detailed analyses need to be done on populations of both species to see if these preliminary results are generally applicable.

The differentiation observed in A.v. suggests that further experiments should be carried out to see if host race formation has occurred in this taxon.

Such host race formation is reported for *A. tsugense* (Smith and Wass 1976). Other studies have demonstrated the existence of intra-specific variation in mistletoes. Variation in morphology and infectivity has been documented in *Phoradendron tomentosum* (D.C.) Engelm. ex. A. Gray subsp. *tomentosum* (May 1971, 1972) and infectivity also varies in *Viscum album* L. (Paine 1950).

In addition, Hawksworth (1961) found evidence of differential infectivity of seeds of *A. vaginatum* in N. Arizona. Seeds from colonies in infected stands of ponderosa pine were used to inoculate both other trees within these infected stands and also trees from adjacent uninfected stands. Inoculations on the former were 10 times as successful as on the latter trees. Ponderosa pine, even when growing in homogeneous stands, can show very high levels of protein (Mitton et al. 1977, Linhart et al. 1981) and monoterpene (Smith, 1964) differentiation over short distances. Hawksworth's results suggest that *A. vaginatum* populations may have adapted to specific features of the biochemical phenotypes of certain stands and are then more successful at recolonizing trees of such phenotypes rather than trees of novel phenotypes. This sort of pattern has been observed in colonization of *P. ponderosa* by scale insects (Edmunds and Alstadt, 1978) and infection of specific varieties of crop plants by specific races of fungi (Barrett, 1983).

#### CONCLUSIONS

These preliminary results indicate that *Arceuthobium* species are amenable to analyses of genetic variability by the use of electrophoretic



methods. This is especially useful in these taxa because of their relative uniformity of morphology at the intra-specific level. In both species analyzed in some detail, given loci appear to have similar levels (i.e. high or low) of polymorphism. The rates of migration of the various allozymes at a given locus are different enough in both these species (and apparently in a third species *A. cyanocarpum*) to enable one to identify samples as belonging clearly to one or another taxon.

There are several polymorphic loci in both species analyzed. In *A. vaginatum*, at least two of these loci shows evidence of heterogeneity of allele frequencies associated with geographic location and with the host species upon which the mistletoe grow.

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# The Anatomy and Morphology of the Endophytic System of *Arceuthobium* spp.<sup>1</sup>

M. Carol Alosi<sup>2</sup> and Clyde L. Calvin<sup>3</sup>

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**Abstract.**--The part of the dwarf mistletoe plant that develops within the host plant body is termed the endophytic system. During establishment of the parasite in new areas of host stem, endophytic cells grow intrusively. After this initial invasion, the development of the endophytic system becomes attuned to the growth form of surrounding host tissues. In host secondary tissues, certain endophytic cells develop a meristem in line with the host cambium. Derivative production is coordinated with host xylem and phloem production, and endophytic cells become integrated with host ray cells to produce a chimera-like unit called an infected ray or sinker. Endophytic tissue of *Arceuthobium douglasii* is hyphal-like in primary host tissues and growth of the parasite is by cell elongation with predominately transverse divisions. Sinkers are produced when the associated host stem converts from primary to secondary growth. The relationship of *Arceuthobium* endophytic cells with host tissues reflects great tissue compatibility and careful evaluation of cytological features may be required to distinguish host from parasite cells. The organisms share a common apoplast, but symplastic continuities are doubtful.

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## INTRODUCTION

The part of the dwarf mistletoe plant that develops within the host has been termed the endophytic system (Thoday and Johnson 1930). There are two fundamentally different endophytic forms--depending on the species of *Arceuthobium*, the host, and perhaps the site of the original infection (Kuijt 1960). These forms produce either localized or systemic infections (Baranyay et al. 1971). The infection forms are usually easily recognized in the field since there are gross differences in the appearance of the branches.

Localized infections are contained within a limited section of the host branch and fusiform swellings, due to hypertrophy and hyperplasia of host tissues and to the presence of the

endophyte, mark the extent of the invasion of the parasite along the host stem. Aerial shoots are limited to the swollen regions and occur in random tufts. As far as is known, all *Arceuthobium* species are capable of developing localized endophytic infections on appropriate host stems (Kuijt 1960).

In contrast to the localized condition, systemic infections are produced only in the case of a few, specific combinations of *Arceuthobium* species and hosts (Kuijt 1960). Instead of being contained within a relatively limited portion of host stem, systemic infections involve entire branches of the affected host, extending into the youngest portions of the shoot, even into the buds (Thoday and Johnson 1930, Parke 1951, Kuijt 1960). Typically there is no swelling, and often infected branches are thin, elongated, and pendulous. Aerial shoots of systemic infections usually show a pattern of emergence related to the age of the host tissue (Kuijt 1960) (fig. 1). The ability to produce systemic infections is the more evolutionarily-advanced condition (Hawksworth and Wiens 1972). Most dwarf mistletoe species do not have the capacity to develop in both primary and secondary host tissues and are typically found in host secondary tissue with a limited capacity to expand vertically in the host branch.

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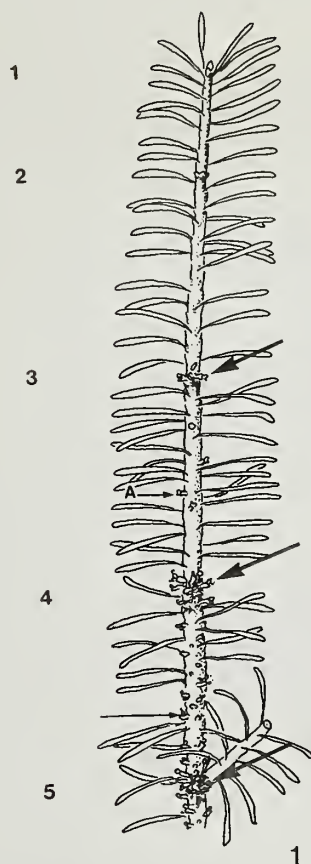


Fig. 1. A diagram of a *Pseudotsuga menziesii* shoot infected with *Arceuthobium douglasii*. The bud scale regions or segment girdles (nos. 1-5) delimit annual growth increments. Although the endophytic system occupies the entire young shoot, the aerial shoots (A) do not erupt until the host stem is 3 years old. Aerial shoots are particularly concentrated at the bud scale regions (large arrows). 1X.

Although it is well established that these two endophytic forms are stable, morphological variations in the genus *Arceuthobium*, the factors which determine form have not been fully appreciated. We believe that the major factor for endophytic form determination is whether the parasite develops within host primary or secondary tissue. Localized infections are a consequence of establishment and proliferation within host secondary tissue, while systemic infections are the result of events that allow establishment and development within host primary tissue. This simple difference has profound morphological implications.

In plant systems primary growth implies a coordinated growth of complex tissues that results in development of the vertical extension of the plant body. Secondary growth involves development of the lateral plant body, expanding the girth of the plant. The fundamental factors that coordinate the growth are not understood but we know they involve intricate processes at the subcellular level which determines cell polarities, cell division planes (Furuya 1984),

and cellulose microfibril orientation (Green 1964; Taiz 1984).

To illustrate these basic morphological forms and to show the relationship to host primary and secondary tissues we will compare the endophytic systems of *A. tsugense* and *A. occidentale*, species which form strictly localized infections, to *A. douglasii*, a species which forms diffuse infections throughout its host's primary and secondary tissues but is also capable of forming localized infections.

## MATERIALS AND METHODS

For light microscope studies of localized infections of *Arceuthobium tsugense* (Rosend.) on western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and *Arceuthobium occidentale* Englem. on digger pine (*Pinus sabiniana* Dougl.) tissue was fixed in FPA (Sass 1958) dehydrated in tertiary butyl alcohol and embedded in paraffin. Twelve-micron-thick sections were stained with ferric chloride/tannic acid/lacmoid (Cheadle et al. 1953). For electron microscopy tissue was fixed in paraformaldehyde-glutaraldehyde and postfixed in osmium. Acetone-dehydrated tissue was embedded in low viscosity resin and sectioned ca. 800 angstroms.

For studies of systemic infections, young stems of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were collected from branches showing dwarf mistletoe (*Arceuthobium douglasii* Englem.) systemic infection. Sections from the first and second intergirdle regions (fig. 1) were diced, fixed and embedded as described above for electron microscopy. Sections were cut at one micron and stained with aqueous 1% toluidine blue O. For low magnification work, older tissue with either localized or systemic infections was soaked in water for 1 hr and then sectioned at ca. 25 microns with a sliding microtome. Individual sections were stained with cotton blue-lactophenol, water rinsed; then stained with phloroglucinol-HCl and mounted directly in glycerol for study and photography.

## OBSERVATIONS

### Localized Infections

A localized infection originates from a primary haustorium at the original infection site. (The infection and establishment process is described by Dr. Knutson in these proceedings.) The primary haustorium gives rise to haustorial strands which extend longitudinally (fig. 2) and sometimes circumferentially (fig. 3) through the host cortex and outer phloem. Radially oriented growths termed "sinker" extend from the haustorial strands into the host secondary vasculature (figs. 2,3,4).

The term "sinker" was first used by Solms-Laubach in 1867 (Srivastava and Esau 1961). The usage in the literature implies reference to a discrete structure. In the green mistletoe



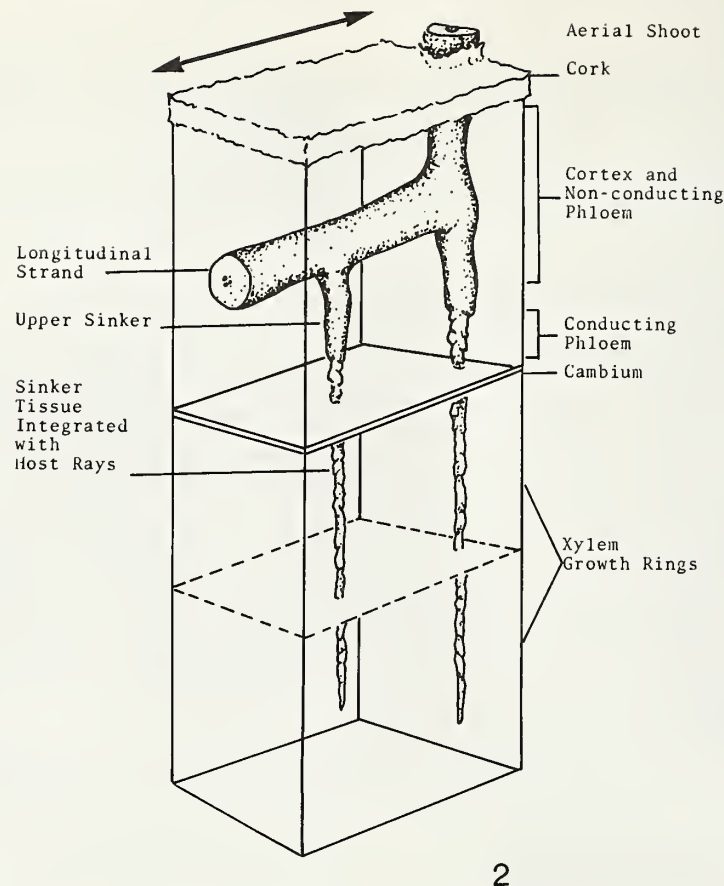


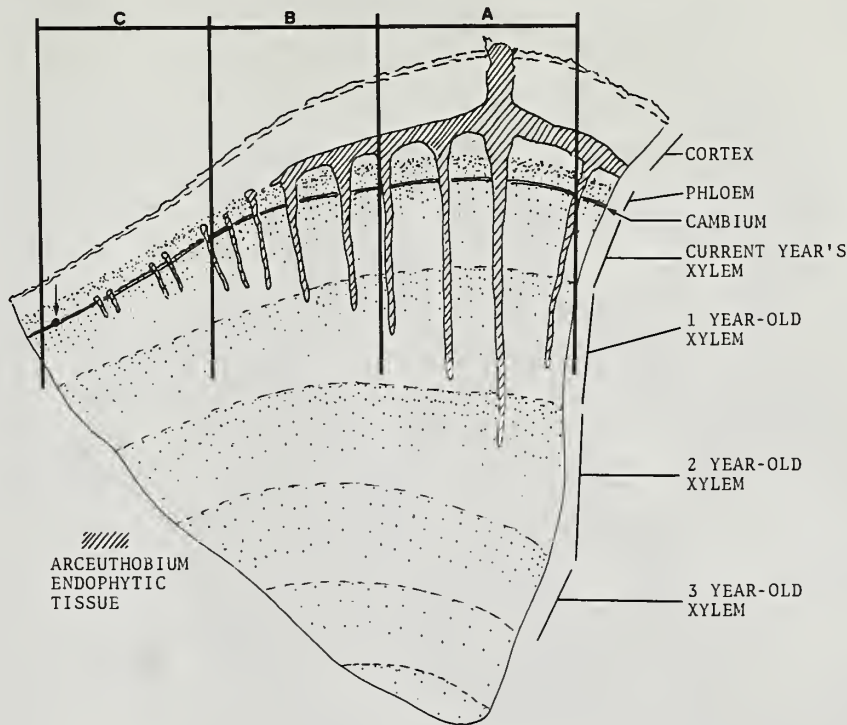
Fig. 2. A diagram of *Arceuthobium tsugense* in *Tsuga heterophylla*. The doubleheaded arrow indicates the longitudinal axis of host stem.

*Phorodendron*, sinkers do appear to be distinct organs of the haustorial system, as the sinkers fit like wedges into the host secondary tissue without intermingling with host cells (Calvin 1967). But, it will be seen that *Arceuthobium* sinkers lose much of their identity as individual structures during maturation of the endophytic system. We use "sinker" as a non-specific term in this report, in reference either to the radially-oriented portions of the endophytic system or to a tissue type.

Investigators differ in their interpretations of the origin and development of sinkers. Some authors report that sinkers originate as lateral protuberances from haustorial strands housed within the host bark. The radially-oriented protuberance of cells grows intrusively towards the host vascular cambium. After reaching the cambial zone the intrusive growth phase of the sinker stops (Kuijt 1960). Another version of sinker origin is that an intrusively growing portion of a longitudinal strand contacts the cambium directly, and there, on the side of the strand, sinkers are initiated

(Alosi 1980). At the contact point a series of cell divisions produces a radial file of sinker cells which becomes embedded in secondary host vasculature (fig. 3). Probably both types of sinker initiation occurs.

There are also differing views on how the radial file of sinker cells comes to be embedded in host xylem while an association with the host phloem is also maintained. Some authors believe that an intercalary meristem is formed near the host cambial zone which provides cells that subsequently become embedded in the host xylem (Thoday and Johnson 1930, Srivastava and Esau 1961). Others have suggested that the intercalary meristem is found at the "neck" of the sinker (where the sinker fuses with the longitudinal strand) (Cohen 1954, Kuijt 1960). And at least one author suggests that two meristems exist--one at the neck, the other at the host cambial zone (Parke 1951). All of these meristematic accommodations to the radial expansion of the host stem may, in fact be found among the various *Arceuthobium* species and their hosts. In tissue we have examined, we see



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Fig. 3. A diagram of *Arceuthobium occidentale* in *Pinus sabiniana*. The depth of the individual sinkers in host growth rings is indicative of the age of the infection in that precise area of the host stem. The oldest portion of the endophytic system shown is in section A, where sinkers extend into two-year-old xylem. The youngest portion is in section C, where the endophyte lies entirely within the cambial and most recently produced phloem and xylem derivatives of the host. Sinkers are initiated within the cambial zone from strand tissue that contacted the host cambium (see arrow, section C).

distinct intercalary meristems juxtaposed to the host cambium (i, fig. 5), but also the arrangement of cells in the neck of some sinkers suggests meristematic activity (arrow, fig. 5).

Sinker tissue is almost always seen in association with host rays (Thoday and Johnson 1930, Cohen 1954, Kuijt 1955 1960, Srivastava and Esau 1961). If young sinkers are originally independent of host ray tissue, new rays or existing ray derivatives are produced in relationship to the little sinker very quickly (fig. 6). The continued conversion of fusiform initials to ray initials adjacent to parasite cells results in the formation of a relatively tall, multiseriate structure consisting of radially-oriented sinker cells more or less surrounded (as seen in sectional view) by host

ray cells (figs. 4, 7).

Coordinated development of sinker tissue and ray tissue perpetuates this chimera-like morphological unit. The structure has been termed an "infected ray" by Srivastava and Esau (1961). Their terminology is appropriate since (as discussed above) the sinker no longer exists as a distinct morphological unit of the *Arceuthobium* endophyte.

#### Anatomy of Infected Rays

As many as five cell types are found in infected rays: host ray parenchyma, host Strasburger cells (=albuminous cells), sinker ground parenchyma, sinker xylem, and sinker sheath cells.



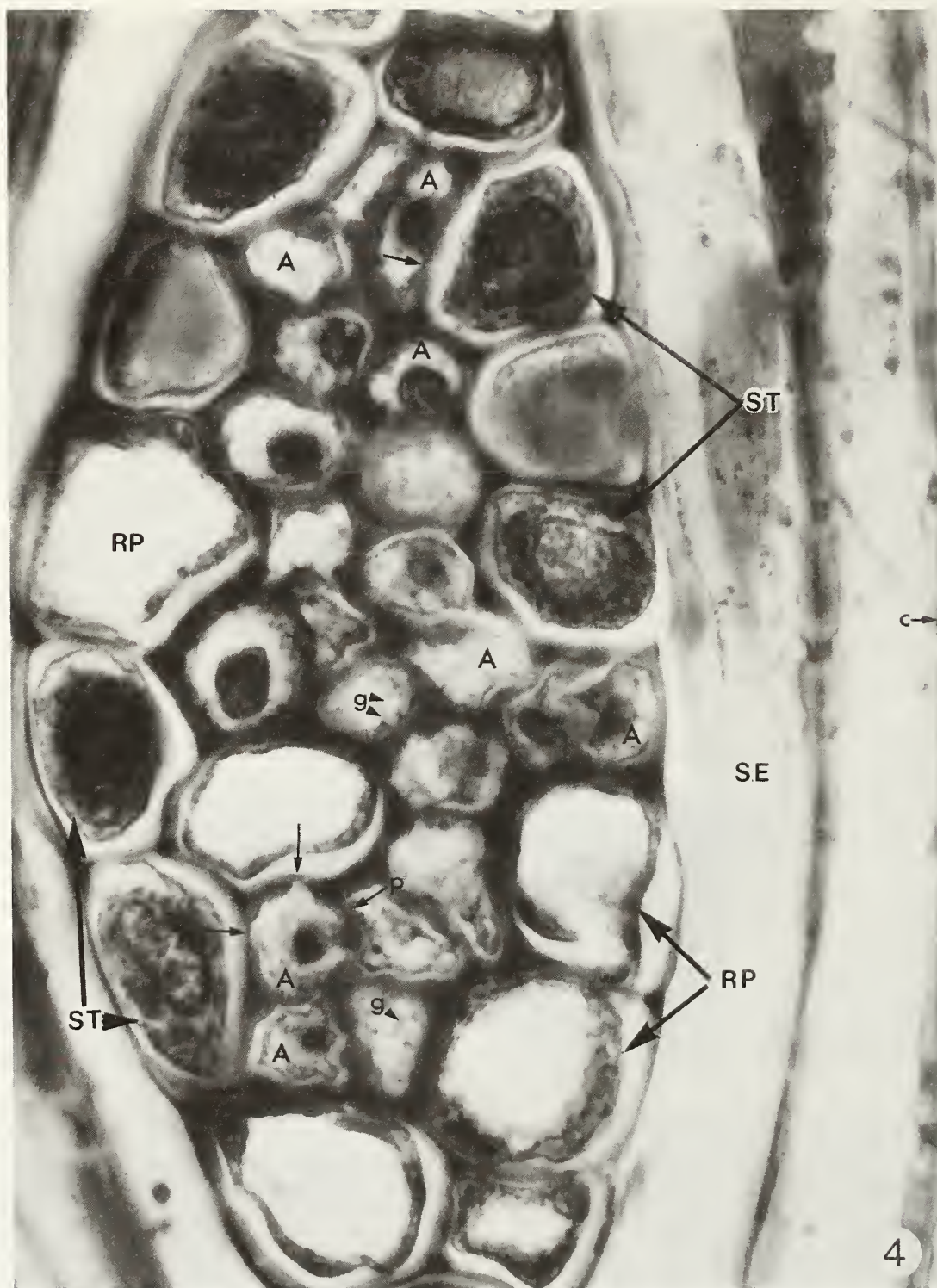


Fig. 4. An infected ray of *Tsuga heterophylla* in the region of functional sieve elements (SE). The smaller *Arceuthobium* sinker cells (A) occupy the central region; they are surrounded by host ray parenchyma (RP) and Strasburger cells (ST). Interspecific pits are at unlabelled arrows. Vacuole-like cavities (g) resulting from the removal of lipids during processing are abundant in sinker cells. c, callose; p, pit between sinker cells. 800X.



Ray Parenchyma: These host cells (RP, figs. 4,6,7) are relatively large cells with an unstained, large, central vacuole. The cytoplasm may contain starch grains and lipid bodies.

Strasburger Cells: Strasburger cells are characterized by connections with lateral sieve areas of adjacent sieve cells. They have dense granular cytoplasm, lack a large, central vacuole and are frequently more upright in orientation than the procumbent ray parenchyma (ST, figs. 4,6).

Sinker Ground Parenchyma: Sinker cells generally have a smaller diameter than host cells in the infected ray, when viewed in tangential section (A, figs. 4,6). However, sinker cells in newly-infected rays, or within the cambial zone, may be similar in size and shape to contiguous host ray cells (fig. 6). The walls of sinker ground parenchyma are usually thick, except near the cambial zone. Fresh sections stained with iodine to detect starch are negative, but lipid stains show the cells packed with large lipid bodies. In paraffin preparations, the lipids are removed and vacuole-like cavities remain (g, fig. 4). Osmium-fixed, resin-embedded material preserves lipids and the droplets are conspicuous in one-micron resin sections when phase-contrast optics are used (l, fig. 6).

Sheath Cells of the Sinker: These cells, of parasite origin, are usually found only in old, infected rays in the outer, non-conducting regions of the host phloem. The sheath cells (sh) are continuous with a layer of sheath cells around the associated longitudinal strand (fig. 5). Sheath cells of sinkers and strands have a large, central vacuole with stainable contents. The cytoplasm contains lipid cavities, and no starch.

Sinker Xylem: Strands of tracheary elements usually are found only in the larger infected rays (x. fig. 7). When xylem is present, a continuous strand may exist through the center of the sinker connecting with the xylem of an associated longitudinal strand, and inwardly with the host xylem. The parasite xylem has helical thickenings which allows extension in relationship to radial stresses. Ultimately, radial extension of surrounding tissue may destroy some of the xylary elements producing lacunae in the center of some infected rays.

Livingston et al. (these Proceedings) discusses hormonal aberrations of dwarf mistletoe infections. Therefore, the implications of abnormal hormone levels on the morphology of infected stems will not be explored here. However, we will point out that in the context of comparing localized and systemic infections, localized infections often produce striking hyperplasia and hypertrophy of xylem which appears to have a rapid onset coincident with the arrival of the sinker tissue in the vascular cambium (fig. 15). Figure 2 illustrates that the

abnormal xylem production is not transmitted evenly around the vascular cylinder, but is localized within a short distance from the parasite sinker tissue. When a localized infection does not circumscribe the host stem, the abnormally wide growth rings will be found only where the sinker tissue is. Cambial cells only a few mm away may produce normal derivative numbers. In old infections, perhaps in response to decline, the growth rings become smaller in width.

#### Function of Sinkers.

Because of juxtaposition of host xylem and phloem with sinkers, the latter are considered to be important in parasite nutrient acquisition. Direct apoplastic continuity through interspecific xylem contacts and cellulose wall free space provides pathways for water and mineral extraction. The parasite also obtains substantial amounts of host-originating photosynthate (Hull and Leonard 1964) and investigators have for many years thought that transfer of organics occurs in the region where the sinkers cross host phloem (Pierce 1905, Weir 1916, Gill 1935, Leonard and Hull 1965). How could these normally symplastically held nutrients be acquired? Direct contact with the host sieve cells is usually found only in small sinkers (figs. 6,7). Sinkers seem more intimately associated with host ray cells and pits occur in the cell walls between the two organisms (arrows, figs. 4). When examined by the electron microscope we find that these pits often have half plasmodesmata (*sensu* Burgess 1972) extending from the host to the parasite. The half plasmodesmata end blindly at the middle lamella (fig. 8). Thus, pits are not indicative of interspecific symplastic continuity via plasmodesmatal connections. Because the organisms are symplastically isolated from each other, we must conclude that in dwarf mistletoe infected stems, the soluble organic nutrients, which are normally held within the symplast of the host, are leaked into the common apoplast of the two organisms. Eventually these nutrients are absorbed into the parasite symplast before being transported into the aerial shoots. We know this, because tracer studies have shown that when the bases of aerial shoots are steam killed, host-fixed carbon compounds are not transported into the shoots, although transpiration continues (Hull and Leonard 1964).

#### Systemic Infections.

Growth in primary tissues--the primary endophytic form.

Kuijt (1960) reported the presence of *A. douglasii* endophytic tissue in Douglas-fir dormant buds. He found that the distribution of parasite tissue in the buds is related to the host procambial strands which develop in relationship to leaf primordia and to a procambial plexus at the base of the bud. When budbreak occurs, cell division and elongation



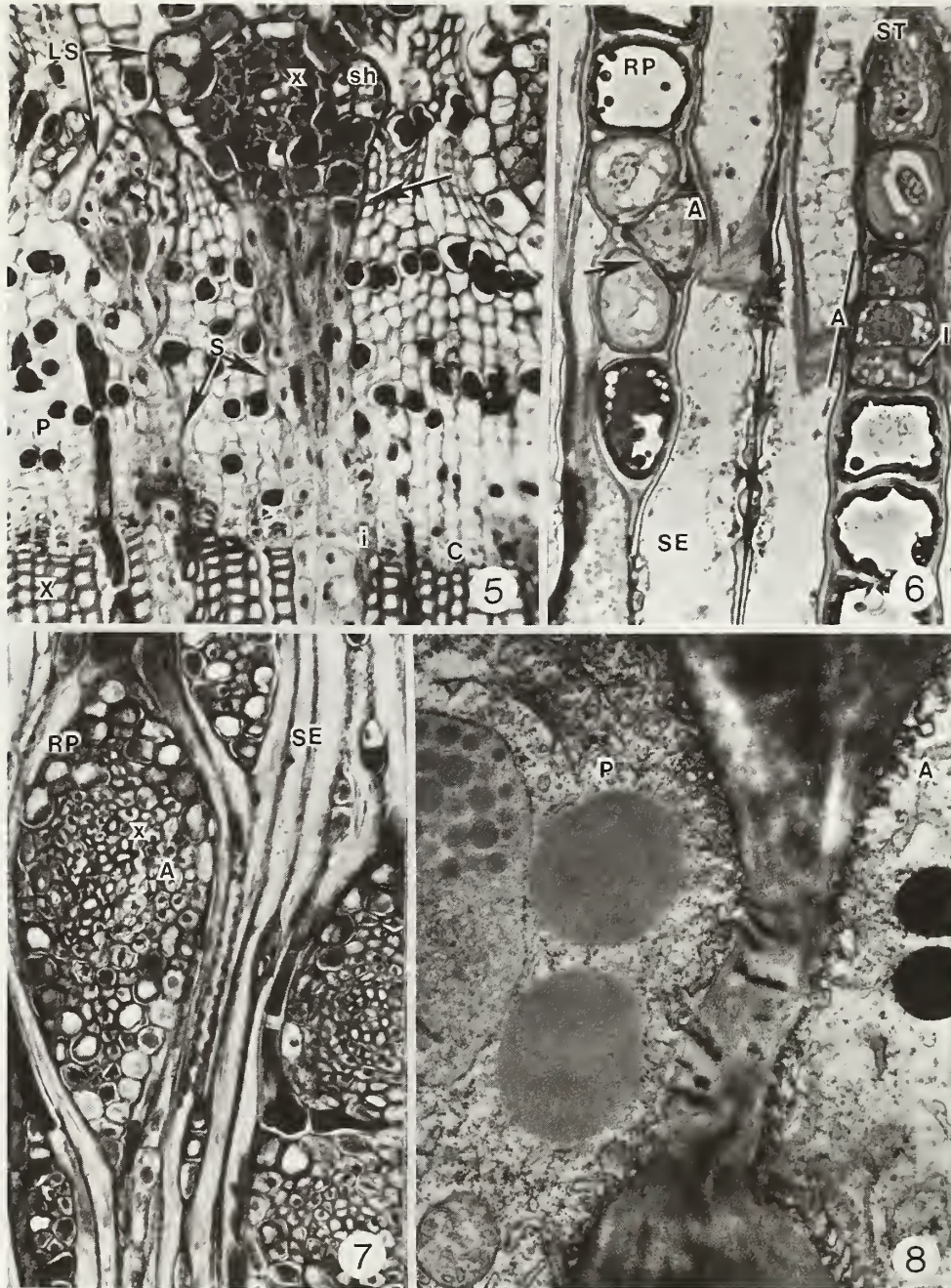


Fig. 5-8. Fig. 5--Infected *Tsuga heterophylla* tissue. Cross section. Longitudinal haustorial strands (LS), one with a central core of xylem (x), connect with sinkers (S). Sinker sheath cells are confluent with the longitudinal strand sheath cells (sh) where a neck meristem (arrow) may adjust the sinker to the radial expansion of the host stem. At the region of the host cambium (C) sinker initials (i) produce derivatives in coordination with xylem (X) and phloem (P) production. 110X. Fig. 6--Young infected rays of *Tsuga heterophylla* in the recently differentiated phloem. Sinker cells (A) and host ray cells have pit connections (arrow). Resin-embedded tissue, phase contrast. 1, lipid droplet; RP, ray parenchyma; ST, Strasburger cell; SE, sieve element. 500X. Fig. 7--Old infected rays of *Tsuga heterophylla* in mature phloem. Sinker tissue (A) is surrounded by host ray cells (RP). Sinker xylem (x) occupies the center of the infected rays. 100X. Fig. 8--Electron micrograph showing a typical pit between *Arceuthobium occidentale* (A) and a *Pinus sabiniana* ray cell (P). The cell wall interface of host and parasite is fused and very thin at the pit. Half plasmodesmata occur on the host side. 30,000X



develops the bud acropetally (Allen 1945) and the associated endophytic tissue develops simultaneously. Our investigation of A. douglasii systemic infections begins with studies of one-year-old stem segments, after the elongating bud stage, when primary growth is well established but before secondary growth begins (fig. 3). We term the parasite tissue associated with primary host tissue the primary endophytic form of systemic infections.

Because of the very close developmental, relationship between host and parasite and the complex assortment of cells in the young shoot, identification of Arceuthobium cells is not always obvious (figs. 9,10). Two cytological details were used to identify parasite cells--nuclear structure and lipid storage bodies.

Arceuthobium has chromocentric nuclei, while Douglas-fir has reticulate nuclear structure (re: Lafontaine 1974). The chromocentric nuclei of A. douglasii have a delicate meshwork of euchromatin throughout the nucleoplasm with dense, heterochromatin masses associated with the nuclear envelope or with the single, sometimes enlarged and vacuolated nucleolus (CN, fig. 11). The reticulate nuclei of Douglas-fir cells (RN) have a conspicuous reticulum of chromatin and lack of heterochromatin masses in the interphase condition (compare RN and CN in figs. 10,11).

The second method of identification is based on the appearance of lipid bodies and is most obvious with phase contrast optics. Arceuthobium cells store lipids in large lipid droplets (l, fig. 11), while Douglas-fir cells store lipids in small spherosomes (s, fig. 11).

The primary endophytic tissue of A. douglasii present within the relatively mature primary host shoot is composed of a complex of mainly uniseriate, hyphal-like strands. The concentration of parasite tissue is greatest in the position of the former procambial plexus, at the girdle-bud scale region. Both host and parasite cells are short, have dense protoplasm, and appear irregularly arranged in this region.

Longitudinal sections of infected year-old stems from the intergirdle region are seen in figs. 9 and 10. The epidermis (e) encases the large, loosely-packed cortical cells (C). Internal to the cortical cells are extremely long, empty-appearing ducts (d) which are lined on their centripetal side by living duct cells (dc). The primary vascular tissue lies to the inside of the duct cells and consists of long, primary sieve cells (SE), shorter Strasburger cells (ST), storage parenchyma with ergastic vacuole contents (st), procambial tissue (or undifferentiated derivatives, pr), and xylem, x). In the center of the young stem is the pith (not shown).

In the intergirdle region some parasite cells are found in neat, longitudinal files within the host primary phloem (fig. 13). It is likely that this tissue is derived from tissue originally associated with the host's procambial strands and it elongated simultaneously with host procambial tissue while undergoing regular transverse divisions. During elongation of the Douglas-fir shoot, the procambium is enlarged by lateral addition to existing procambial strands. Arceuthobium douglasii appears to associate with this additional vasculature by hyphal-like, tortuous extension of endophytic tissue through the cortex (A, fig. 9, 13) with subsequent deflection towards the cambial cylinder and intrusive growth into the host phloem and procambium (fig. 12).

In contrast to the arrangement of cells in localized infection, no sinkers or multiseriate haustorial strands develop in the primary endophytic form of A. douglasii. Only the inner cortex, the phloem, and the procambium are invaded. The parasite cells of primary endophytic tissue all appear to be the same cell type--no specialized cells differentiate in relationship to host phloem and no tracheary elements form. However, as in the infected rays of secondary tissue, pits are frequently found between parasite and juxtaposed host cells of the primary plant body (unlabelled arrow, figs. 10,11).

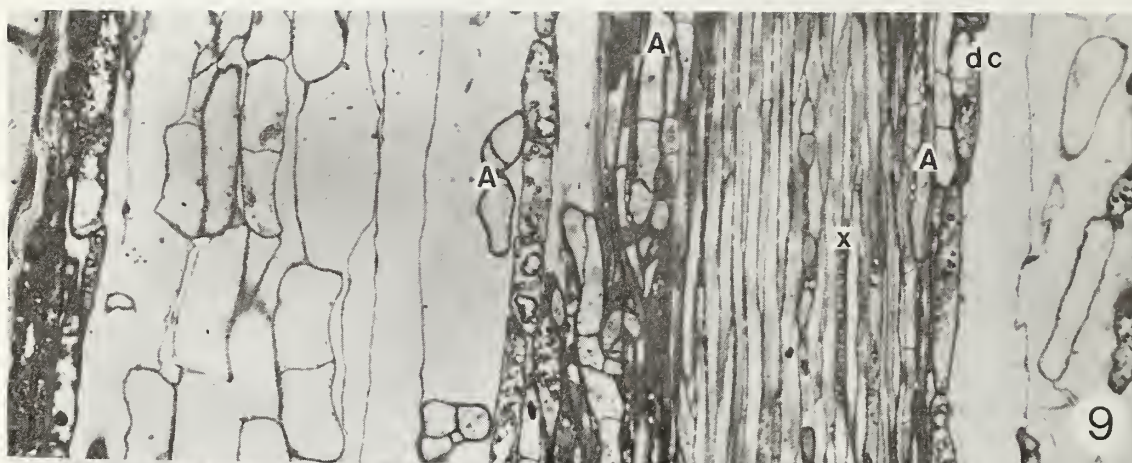
Growth in secondary tissues--the secondary endophytic form.

The beginning of secondary growth is marked by formation of the vascular cambium and a shift from axillary development to lateral growth and differentiation. The predominately uniseriate strands of A. douglasii established during development in the host primary shoot, begin the transition to the more complex, multiseriate, longitudinal strands of the secondary endophytic form by ceasing elongation, expanding radially, and undergoing periclinal division (unlabelled arrow, fig. 14).

The host vascular cambium appears to have a growth polarizing effect on endophytic tissue. Apical cells of hyphal-like strands respond by intrusively growing towards the cambium. Other cells, in intercalary positions of hyphal-like strands of the primary endophytic system, develop outgrowths that extend toward the vascular cambium (S, fig. 14). Growth is intrusive among medullary rays, declining tiers of secondary phloem derivatives, and newly formed ray cells (R, fig. 14). These radially oriented strands of Arceuthobium cells will become the sinkers of the secondary endophytic system within the secondary host tissue.

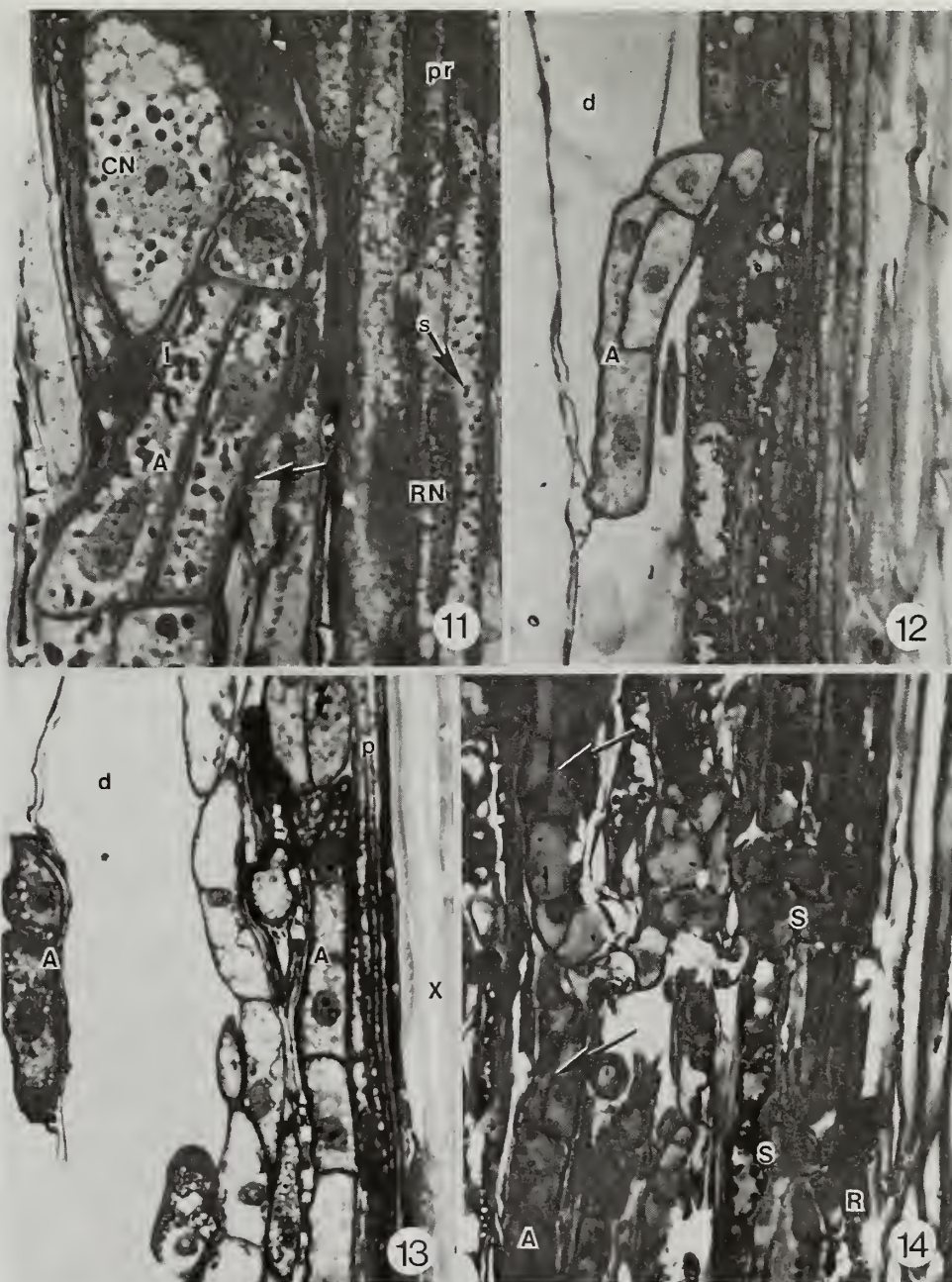
The basic structure of A. douglasii endophytic tissue of systemic infections in secondary vasculature is the same as that established in localized infections, i.e., haustorial strands, and sinkers. As in localized





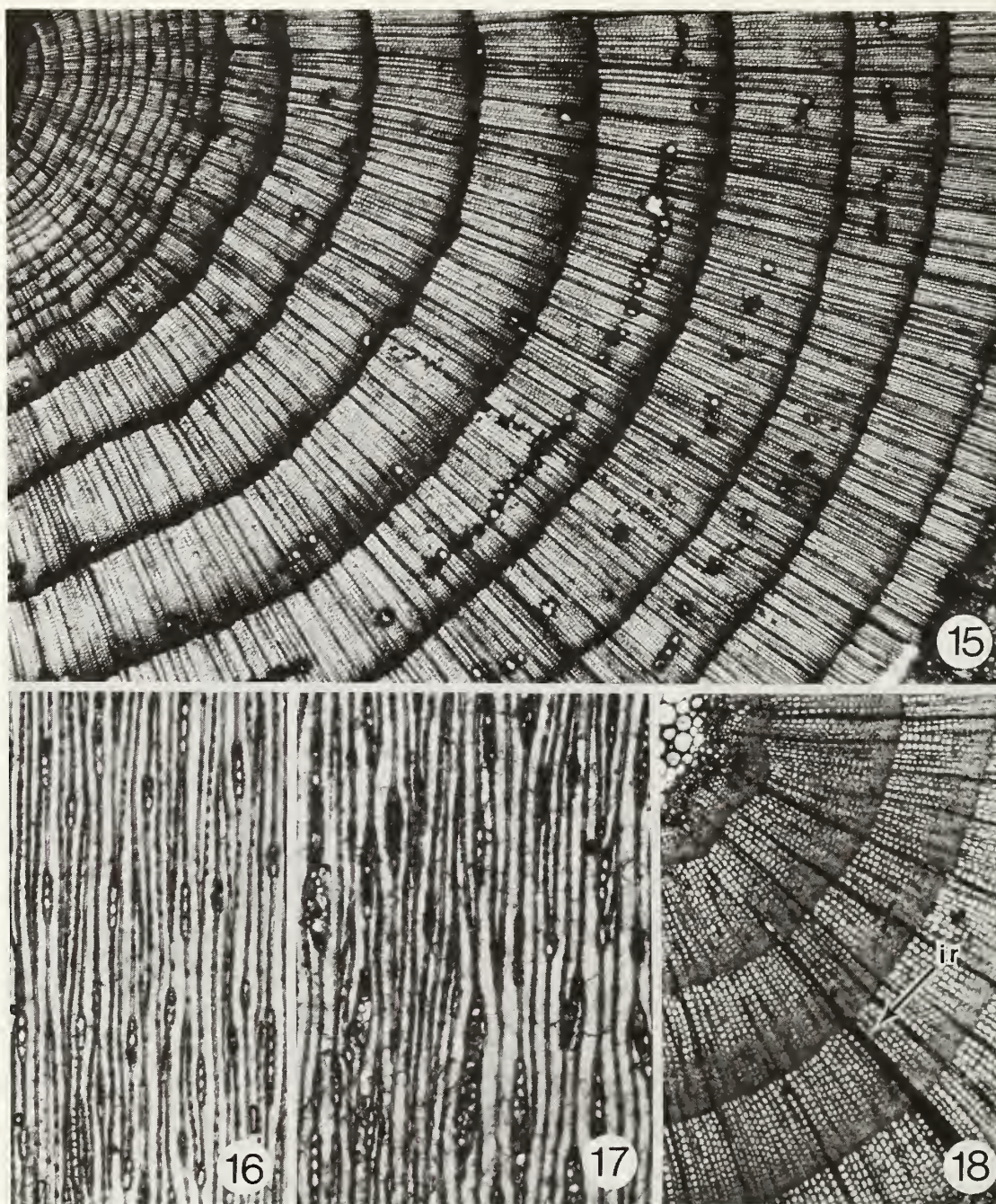
Figs. 9 and 10. Tangential section infected primary shoot of *Pseudotsuga menziesii*. Fig. 9--Strands of the primary endophytic system of *Arceuthobium douglasii* (A) are found in the inner cortex (C), primary phloem (p); d, duct; e, epidermis; pr, procambial tissue. 50X. Fig. 10--Hyphae of the primary endophyte (A) is integrated into the primary phloem of the host. The reticulate nuclei of the host (RN) is distinct from the chromocentric nuclei of the parasite (CN). SE, sieve element; st, storage parenchyma; ST, Strasburger cell. 200X.





Figs. 11-14. *Arceuthobium douglasii* in Douglas-fir primary tissue. Longitudinal sections; 1 micron thick. Fig. 11--Parasite cells (A) with large lipid droplets (l) and characteristic chromocentric nuclei (CN) are associated with host phloem and procambial tissue (pr) which have small spherosomes (s) and reticulate nuclei (RN). 500X. Fig. 12--A hyphae of *Arceuthobium* tissue (A) within the ducts (d) of the host shoot appears to be invading host vascular tissue by intrusive growth. 170X. Fig. 13--Hyphal strands of the parasite (A) are longitudinally oriented within the primary phloem of the host (p) and do not extend into the xylem (X). Hyphal strands also grow intrusively between ducts (d). 150X. Fig. 14--The transition of the primary endophytic form to the secondary endophytic form seen in radial section. Radial expansion of hyphal strand (A) and periclinal division (arrow) of cells marks the first stage in conversion of the hyphal strand to the multi-seriate haustorial strands of the secondary endophytic form. Sinkers, found only in the secondary endophyte, form from radially-oriented protrusions (S) which grow intrusively through newly formed rays (R). 175X.





Figs. 15-18. Infected and uninfected wood of *Pseudotsuga menziesii*.  
 Fig. 15--Cross section. Stem with localized infection of *Arceuthobium douglasii*. The outer 9 rings show hypertrophy which is characteristic of localized infections. The smaller, inner rings were produced prior to infection. 20X. Fig. 16--Tangential section. An inner, uninfected growth ring from tissue shown in Fig. 15. Small, normal, uniseriate rays are found. 50X. Fig. 17--Tangential section through an outer, hypertrophied ring from tissue shown in Fig. 15. Rays are abnormally wide due to the presence of *Arceuthobium* cells within the rays. 50X. Fig. 18.--Cross section of stem having systemic infection symptoms. Uniform growth rings are present despite the presence of the parasite. Infected rays are narrow except those that formed from medullary rays (ir). 35X.



infections the secondary endophytic form may have ground tissue, xylem, and sheath cells (Parke 1951).

There are no localized swellings in secondary host tissue with systemic infections because the secondary endophytic form develops from the pre-existing primary endophytic system, and the conversion to the secondary endophytic form occurs more or less simultaneously throughout the entire stem segment. There is apparently less anomalous wood structure in systemic infections, perhaps as a consequence of the continual association of parasite and host tissues. Growth rings are of a consistent size and traumatic resin ducts, present in localized infections, are not seen in the wood of A. douglasii systemic infections (compare figs. 15 and 18). The pendulous growth habit of systemically-infected Douglas fir branches is a consequence of abnormal elongation during primary growth.

#### CONCLUSION

The dwarf mistletoes are remarkably attuned to host growth, whether primary or secondary. Because of the parasite's sensitivity to the developmental processes of its host, Arceuthobium species that form systemic infections keep pace with the longitudinal growth of the host stem by hyphal-like extensions of a diffuse, primary endophytic system. Later, in areas where the host's primary growth stops and conversion to secondary growth occurs, the associated dwarf mistletoe tissue morphologically adjusts in accommodation to the new host growth form. Parasite growth in secondary host tissues in all Arceuthobium species, whether in systemic or localized infections, is characterized by the development of radially oriented sinkers. By coordinating derivative production with host cambial activity, sinkers become embedded in host xylem and remain intimately associated with the annual phloem increment. Sinkers lose their identity as individual morphological structures very quickly after their initiation. They become integrated into rays to form the chimera-like infected rays.

The function of sinkers is presumed to be both nutrient absorption and facilitation of nutrient transport out of the host vasculature. How this process occurs is not clear since the dwarf mistletoes have no phloem even in the aerial shoots. (Relic sieve elements have been discovered by Calvin (in press) which are associated with fruit in a primitive Arceuthobium species). The total absence of phloem is an anatomical feature shared by few other vascular plants and the loss of this fundamentally important tissue is a physiological adaptation which we do not understand.

Studies on the morphology of the encophytic systems of Arceuthobium have illucidated the

highly developed accomodation of the parasite to the host anatomy and physiology. This is demonstrated most strikingly in systemic infections where both the primary and secondary host tissue is parasitized and growth is closely coordinated. Anatomical studies have shown that interspecific symplastic union does not occur. Furthermore, interspecific xylary connections with a continuous haustorial xylary system is not necessarily established throughout the endophytic system. (In fact, in the primary endophytic system of A. douglasii no xylem is differentiated and contact with host xylem does not generally occur). The physiological implication of these morphological facts is that the cellulosic cell wall portion of the free space continuum which joins host and parasite is in all probability the major conduit for interspecific nutrient flow, as well as being important in water conduction. We know, however, that eventually living parasite cells are involved in the transport of organic nutrients into the aerial shoots (Hull and Leonard 1964), but the precise route and rate of transport is unknown.

These facts may be helpful in developing chemical control methods for dwarf mistletoes. In order to select systemic pesticides for specific needs, it is helpful to understand both the translocation characteristics of a compound, and whether the target pest is associated with the host's symplast, apoplast, or both. The apparent importance of the free space continuity in dwarf mistletoe/host nutritional relationships might well be exploited in development of control measures.

It has been twenty years since Leonard and Hull published their pioneering tracer experiments in host/mistletoe nutritional relations. Unfortunately, very little similar work has been done since. In the future one hopes that sugar transport rates into aerial shoots will be determined. This information is essential for further understanding of transport phenomenon in aphloic tissue such as the dwarf mistletoes. In addition, tracer work which allows microautoradiography of diffusible substances will be most valuable, allowing visualization of transport paths between host and parasite and within the dwarf mistletoe endophytic system and aerial shoot tissue. Such precise knowledge of interspecific nutrient transport will be fundamental to research associated with effects of exogenous substances on the mortality of the parasite.

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# Altered Concentrations of Absciscic Acid, Indole-3-Acetic Acid, and Zeatin Riboside associated with Eastern Dwarf Mistletoe Infections on Black Spruce<sup>1</sup>

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**Abstract.**--Noninfected stem segments of black spruce (*Picea mariana*), and stem tissue infected with eastern dwarf mistletoe (*Arceuthobium pusillum*) were analyzed monthly from April to October, 1982, for absciscic acid (ABA), indole-3-acetic acid (IAA), zeatin, and zeatin riboside. High performance liquid chromatography was used to purify all plant extracts and to ascertain concentrations of IAA, zeatin, and zeatin riboside. ABA levels were analyzed using gas chromatography. Zeatin was not detected while zeatin riboside was detected only in samples collected in April-June. Concentrations of zeatin riboside (up to 65 ng/g fresh wt) were higher in infected tissue in 15 of 18 comparisons. IAA concentrations (28-479 ng/g fresh wt) were higher in 15 of 16 comparisons from April and May samplings. Impurities in the samples prevented adequate analysis of IAA from June to October. ABA levels (37-1546 ng/g fresh wt) were consistently lower in infected tissue ( $P(T) < 0.1$ ) throughout the sample period. Alterations in plant growth substances are apparent in successful infections of dwarf mistletoe.

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## INTRODUCTION

Dwarf mistletoes (*Arceuthobium* spp.) can alter normal growth of conifers as indicated by (1) proliferation and elongation of tree shoots (Tinnin and Knutson 1980), (2) swelling of the infected stem tissue, and (3) reduction in tree growth that may lead to death (Hawksworth 1978).

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The reduction in growth occurs because dwarf mistletoe does not supply all of the photosynthetic assimilates it uses (Miller and Tocher 1975) and obtains assimilates from its host (Hull and Leonard 1964), i.e. it acts as an assimilate sink. The parasite is thought to induce these changes in its host by altering the balance of plant growth substances in the tree tissue, particularly cytokinins (Knutson 1979). Studies (Paguet 1979, Schaffer et al. 1983) have shown higher concentrations of cytokinins (zeatin and zeatin riboside) or cytokinin-like activity in tissue infected with dwarf mistletoe than noninfected tissue.

Other plant growth substances are known to affect source-sink relations in plants (Wareing and Patrick 1975) but have not been analyzed for dwarf mistletoe infections. A better understanding of how dwarf mistletoe infections affect other plant growth substances besides cytokinins is necessary to ascertain how this parasitic plant is able to obtain assimilates from its host. This study compared the amounts of indole-3-acetic acid (IAA), absciscic acid (ABA) as well as zeatin and zeatin riboside associated in black spruce (*Picea mariana* (Mill.) B.S.P.) tissue infected by eastern dwarf mistletoe (*A. pusillum* Pk.) vs. noninfected tissue.

## MATERIALS AND METHODS

**Tree Material.**--Black spruce tissue infected with eastern dwarf mistletoe was obtained from an uneven aged stand (tree age up to 120 yr) on the Fond du Lac State Forest, Carlton Co., MN. Tissue for plant growth substance analysis was collected during the third week of each month from April through October, 1982. Trees were selected by establishing a 2 m wide transect in a random direction through the stand. From the trees whose stems were within the transect and whose crowns were dominant or codominant in the overstory, four trees with male infections of eastern dwarf mistletoe and four trees with female infections were selected at random. A new tree sample was obtained for each month of tissue collection. The branch with the dwarf mistletoe infection was tagged as well as a noninfected branch originating from the same node on the tree stem. Branch segments that had formed one and three years previously were cut from the two branches and placed on dry ice. Sampling occurred during mid-day. Needles, buds, and new growth were removed, the segments were cut into 1-5 mm pieces, and the tissue was immediately frozen until extraction.

**Extraction.**--Extraction took place in a cold room at 1 to 2 C. Within 24 hr of collection, one gram of the sliced tissue segments were placed in 50 ml of 80% methanol containing 10 mg/l butylated hydroxy-toluene (Calbiochem, LaJolla, CA) and homogenized with a Polytron homogenizer (Brinkman Instruments) for 5 min at 1 C. Prior to homogenizing, ca. 5,000 dpm of [ $^{14}\text{C}$ ]IAA (52 mCi/mmol, Amersham) and ( $\pm$ )[ $^{14}\text{C}$ ]ABA (17.9 mCi/mmol, Amersham) were added to each sample to quantify the recovery efficiency. After homogenizing, the samples were placed overnight on a shaker at 1 C. On the following day the samples were filtered, reduced to aqueous phase *in vacuo* at 30 C, frozen and thawed, and then centrifuged at 15,000 g. The supernatants were then reduced to dryness *in vacuo* at 30 C and stored at -20 C.

**Purification.**--Samples were purified using high performance liquid chromatography (HPLC) as described by Schussler et al. (1984) and Hoover (1982). Samples were resuspended in 4 ml water, filtered, and injected into a 2-column HPLC system consisting of PRP-1 (a porous polystyrene gel) and RSiL. The mobile phase for the first column was a pH/ethanol gradient and for the second column an ethanol gradient. Based on elution times for standards, separate fractions were collected for the cytokinins, IAA, and ABA. Additional purification of the cytokinins and IAA was done with a 1-column HPLC using Nucleosil and 0.1 N acetic acid/acetonitrile mobile phase. Zeatin and zeatin riboside were separated at this time.

**Quantification.**--Zeatin and zeatin riboside were quantified using HPLC with a cation exchange column and two UV detectors connected in series monitoring at 254 nm and 210 nm (Hoover 1982). IAA was quantified using an anion exchange column

and two different detectors (fluorescence and oxidative amperometric) connected in series (Hein et al. 1984). Amounts of the compounds were estimated by comparing peak heights of the samples to those of an external standard curve. Ratios of peak heights as measured by the two different detectors were calculated for each sample and were compared to the mean ratio of the standards. If the ratio of a sample was more than 2 standard deviations from the mean ratio of the standards, the peak of the sample was considered impure. After HPLC analysis, the IAA fractions were analyzed for radioactivity using liquid scintillation spectrometry to calculate the loss of [ $^{14}\text{C}$ ]IAA during purification and quantification.

ABA fractions were methylated and then quantified using gas liquid chromatography with an electron capture detector (Schussler et al. 1984). The methylated samples and standards of ABA were brought up in 500  $\mu\text{l}$  of ethyl acetate containing the ethyl ester of ABA as an internal standard. One  $\mu\text{l}$  was injected (1:10 split ratio) into the capillary column. Quantification was based on the ratio of the methyl ester peak height to the ethyl ester peak height. The remaining volume in the ABA samples was analyzed for radioactivity of ( $\pm$ )[ $^{14}\text{C}$ ]ABA.

Authentication of zeatin riboside and IAA was accomplished using gas chromatography-mass spectrometry (GC-MS). Mass spectra were obtained using a KRATOS MS-25 GC-MS operating at an ionization potential of 70 eV with a DS-55 data system. Cytokinins were derivitized at 55 C for 0.5 h with N,O-bis (trimethylsilyl)trifluoroacetamide-trimethylchlorosilane (99:1, 5  $\mu\text{l}$ ; Regis Chemical Co., Morton Grove, IL) with 5  $\mu\text{l}$  of pyridine as solvent. The GC-MS ran with a temperature programmed from 160-280 C at 40 C/min, flow rate 2 ml/min. A 10 m methyl silicone fused silica column (0.53 mm dia.) (Hewlett Packard) was used. IAA samples were derivitized with 6  $\mu\text{l}$  BSFTA (Regis Chemical Co., Morton Grove, IL) for 0.25 h at 50 C and were run on the GC-MS with a temperature programmed from 60-240 C at 40 C/min. Samples were run on a 25 m cross-linked 5% phenyl methyl silicone fused silica column (0.3 mm dia.) (Hewlett Packard).

To avoid the assumptions of normal distribution and equal variances, nonparametric statistical tests were used as described by Conover (1980). To reduce the variation in concentrations of plant growth substances due to sampling different trees, observations made on the same tree were paired for statistical analysis.

## RESULTS

**Zeatin and Zeatin Riboside.**--No peaks occurred in the fraction that eluted with the zeatin standard during HPLC analysis, and therefore this compound was not detected in any of our samples. Small amounts of zeatin riboside were detected only in the first three sample



months. During analysis, the only peaks that appeared in the fraction which corresponded with the elution time of the zeatin riboside standard were judged to be pure zeatin riboside. GC-MS of the samples showed mass spectra characteristic of TMS-zeatin riboside (Dauphin et al. 1977). Nondetectable amounts of zeatin and zeatin riboside were 5 ng/g fresh wt or less. Losses during purification and analysis were not computed.

In the April samples, amounts of zeatin riboside were larger from infected tissue than from noninfected tissue in 5 of 6 pairings (one yr old branch segments: 24,15; 14,<5; 10,9; 25,17; 10,18 ng/g fresh wt. Three yr old branch segments: 8,<5 ng/g fresh wt). In the May samples, zeatin riboside values were higher in 4 of 6 pairings (one yr old branch segments: 65,<5; 11,<5; 11,<5 ng/g fresh wt. Three yr old branch segments: <5,39; 12,<5; <5,12 ng/g fresh wt.). In the June samples, amounts were larger in 6 of 6 pairings (one yr old branch segments: 16,<5; 14,<5; 53,<5; 13,<5; 13,<5 ng/g fresh wt. Three yr old branch segments: 10,<5 ng/g fresh wt.).

**IAA.**--IAA was detected in our samples throughout the growing season (fig. 1), but most samples collected after May were judged to have impure IAA peaks during HPLC analysis and were excluded from further consideration. GC-MS of the samples showed mass spectra characteristic of bis-TMS-IAA (McDougall and Hillman 1978). The amounts of IAA detected in our samples were corrected for losses of [ $^{14}$ C]IAA which averaged 35% recovery for April, 30% for May, and 17% for the remaining months. In April, all ten possible pairings of infected and noninfected branch segments had higher concentrations of IAA in the infected tissue. Five out of the possible six pairs in May had larger amounts of IAA associated with dwarf mistletoe infection. For the time period after May, only three out of the possible nine pairs had higher concentrations of IAA in the infected tissue. Because of the large number of missing values, statistical tests were not completed on this data set.

**ABA.**--Compared to the other plant growth substances analyzed, large amounts of ABA were detected in the samples (fig. 2). Amounts of ABA were corrected for the losses of [ $^{14}$ C]ABA, and the recoveries averaged 66%, 71%, 77%, 61%, 72%, 73%, and 69% for the respective sampling months of April through October.

For each grouping of branch segments, ABA concentrations for male and female infections did not differ (Mann-Whitney test,  $P(T)>0.1$ ) except for one yr old branch segments in October ( $P(T)=0.05$ ). This difference was probably due to the variation of ABA levels observed for this month. Therefore, branch segments from male and female infections of eastern dwarf mistletoe were pooled for the remaining analyses.

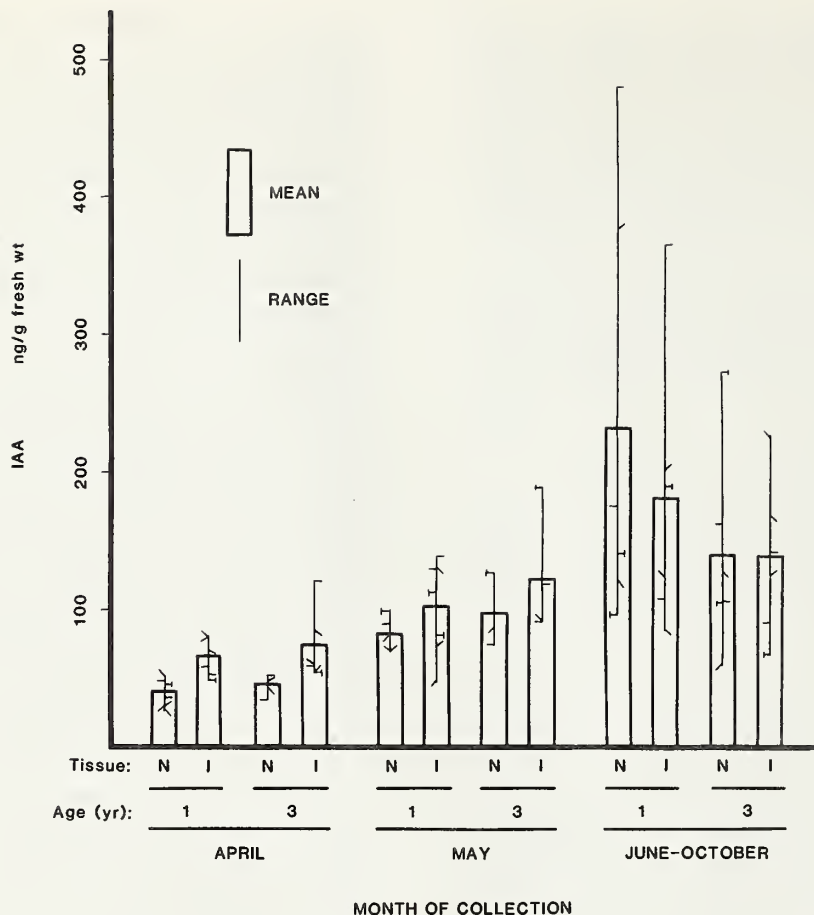
Additional statistical analyses were done using differences of paired observations; the ABA concentration of the infected branch segment was

subtracted from the ABA concentration of the corresponding noninfected branch segment on the same tree. The size of the differences between infected and noninfected tissue did not differ between one yr old branch segments and three yr old branch segments for April, July, and October (Wilcoxon Signed Ranks Test,  $P(T)>0.1$ ), but differences in the concentrations of ABA between infected and noninfected tissue were larger in one yr old branch segments for the other months ( $P(T)<0.05$ ). Therefore, additional statistical tests were calculated separately for the two different branch segments. The size of the differences in ABA concentrations between infected and noninfected tissue were significantly different between months (Kruskal-Wallis Test,  $P(X)<0.05$  for one yr old branch segments and  $0.05<P(X)<0.10$  for three yr old branch segments). Therefore, values for each month were not pooled, and calculations for the significance of the differences between infected and noninfected tissue were derived for each branch segment within each month. These differences in ABA concentrations between infected and noninfected tissue were significant for each combination of month and branch segment (Wilcoxon Signed Ranks Test,  $0.05<P(T)<0.1$  for three yr old branch segments in October,  $P(T)<0.05$  for the remaining combinations).

Differences in ABA concentrations between one yr old branch segments and three yr old branch segments for infected tissue were significant only in April (Wilcoxon Signed Rank Test,  $P(T)<0.05$ ) and in July ( $0.05<P(T)<0.1$ ). In contrast, ABA concentrations were significantly larger ( $P(T)<0.05$ ) in one yr old branch segments than the three yr old branch segments for noninfected tissue for each month except April and October ( $P(T)>0.1$ ).

## DISCUSSION

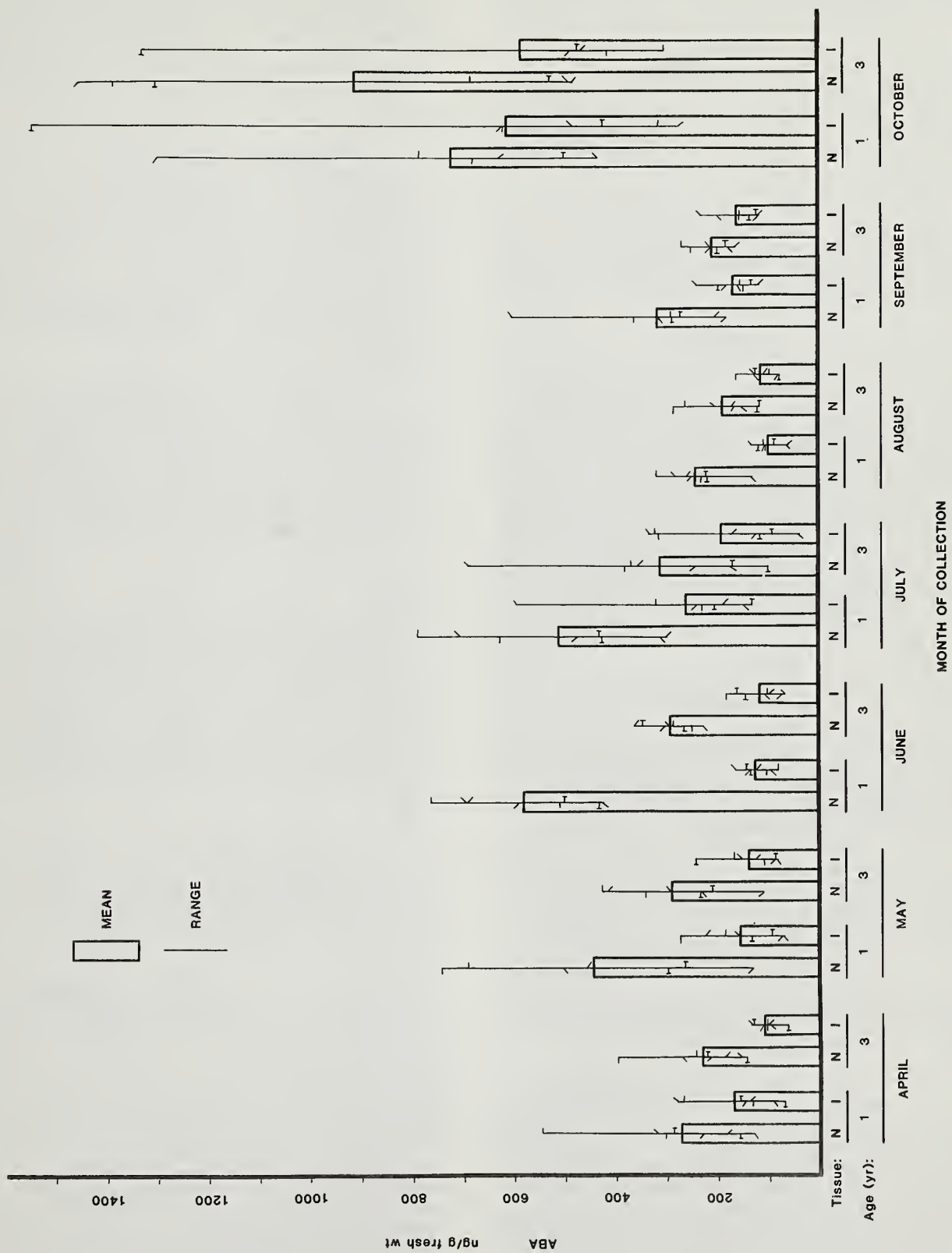
**Zeatin and Zeatin Riboside.**--There was more zeatin riboside in spruce tissue infected with eastern dwarf mistletoe than noninfected tissue. This confirms previous observations of increased cytokinin activity associated with dwarf mistletoe infections (Paquet 1979, Schaffer et al. 1983). Zeatin was not detected in any samples but still may be present in black spruce tissue. Paquet (1979) found zeatin in stem tissue of Douglas-fir (*Pseudotsuga menziesii*) infected with *A. douglasii* and in stem tissue of western hemlock (*Tsuga heterophylla*) infected with *A. tsugense*. Zeatin was not detected in noninfected tissue of these two conifers and was detected less frequently than zeatin riboside in the infected tissues. Zeatin riboside was rarely detected in our samples (22 of a possible 224). Twenty of the 22 concentrations were below 25 ng/g fresh wt. Apparently the amounts of zeatin riboside extracted from conifer tissue are small as observed in our study with black spruce and in the study of Little et al. (1979) with balsam fir (*Abies balsamea*, 5 ng/g fresh wt for the cambial region). Also, zeatin riboside was most prevalent



**Figure 1.**--Amounts (ng/g fresh wt) of indole-3-acetic acid in noninfected branch segments and branches infected with eastern dwarf mistletoe on black spruce. Four different hatch marks († ‡ ¶ §), representing data points, on the left side of the range bars represent male infections, right side hatch marks represent female infections. The same type of hatch mark on the same side of the four range bars in a given month represent samples obtained from the same tree. Missing hatch marks indicate missing values. N=noninfected, I=infected, 1=branch segment formed 1 yr previously, 3=branch segment formed 3 yr previously.

**Figure 2.**--(following page). Amounts (ng/g fresh wt) of abscisic acid in noninfected branch segments and branches infected with eastern dwarf mistletoe on black spruce. Four different hatch marks († ‡ ¶ §), representing data points, on the left side of the range bars represent male infections, right side hatch marks represent female infections. The same type of hatch mark on the same side of the four range bars in a given month represent samples obtained from the same tree. Missing hatch marks indicate missing values. N=noninfected, I=infected, 1=branch segment formed 1 yr previously, 3=branch segment formed 3 yr previously.





in the spring and confirms previous observations made on trees during this time of year (Alvim et al. 1976, Hewett and Wareing 1973, Paquet 1979).

Observed increases in zeatin riboside associated with dwarf mistletoe infection is consistent with the hypothesis that increased cytokinin activity is associated with growth changes that occur with dwarf mistletoe infection (Knutson 1979, Paquet 1979, Schaffer et al. 1983) and with growth changes that occur in other plant diseases (Dekhuijzen 1976, Sequeira 1973). Cytokinins have been implicated in controlling the outgrowth of lateral buds (Phillips 1975), and therefore are probably involved in the loss of apical dominance and the resulting witches' broom formation associated with dwarf mistletoe infection. Cytokinins also promote cell division associated with gall formation of other diseases (Dekhuijzen 1976, Sequeira 1973) and could be involved with the stem swellings associated with dwarf mistletoe infection. The mobilization of nutrients to areas of the leaf with higher cytokinin concentrations results in green island formations are commonly observed with leaf parasites (Dekhuijzen 1976, Sequeira 1973). Cytokinins could be involved with the increased partitioning of nutrients to dwarf mistletoe infected tissue. However, it was concluded with another parasitic plant, dodder (*Cuscuta* spp.), that cytokinins were not involved in the partitioning and uptake of nutrients from the host to the parasite (Jacob et al. 1975).

**IAA.**--IAA concentrations in April and May were consistently higher in black spruce tissue infected with dwarf mistletoe than noninfected tissue. This trend did not continue for the remaining sample periods, but this could be due to the low number of quantifiable samples. More data are needed to ascertain how IAA concentrations vary in dwarf mistletoe infections during the growing season. The concentrations of IAA observed in this study (28-479 ng/g fresh wt) were lower than those measured in other conifer tissue (1000-2000 ng/g dry wt for sitka spruce (*Picea sitchensis*) shoots (Little and Wareing 1981) and 5-720 ng/g fresh wt for Douglas-fir shoots (DeYoe and Zaerr 1976b)). Such differences could be due not only to tree species differences but also to different extraction techniques. Our recoveries of IAA (9.8 to 34.7%) from extracts of conifer tissue were higher than those obtained in other studies (5%, DeYoe and Zaerr 1976; 5-27%, Little et al. 1978).

IAA is closely associated with the control of apical dominance which involves inhibiting the growth of lateral buds and the directed transport of nutrients (Phillips 1975). The increased concentrations of IAA we observed in dwarf mistletoe infections could disrupt the natural balance of IAA in tree stems with the resulting disruption of apical dominance and witches' broom formation. IAA also is associated with cambial activity (Little and Wareing 1981) and with gall formation of other diseases (Pegg 1976a, Sequeira 1973) and could be involved along with cytokinins in the stem swelling associated with dwarf mistletoe infections.

**ABA.**--The concentrations (37-1546 ng/g fresh wt), monthly variations in concentrations, and recoveries (61-77%) of ABA are similar to those found for shoots of other conifers (Andersson et al. 1978, Webber et al. 1979). When values are compared within trees which were sampled, ABA concentrations were consistently lower in black spruce tissue infected with dwarf mistletoe.

Increases in concentrations of ABA are associated with tissue dormancy, water stress (Milborrow 1974, Walton 1980), and with stunting and wilting of diseased plants (Pegg 1976b). Conversely, reduction in concentrations of ABA are associated with the initiation of bud (Dörfling 1976, Dumbroff et al. 1979) and shoot growth (Webber et al. 1979) in the spring and could have a role in apical dominance (Phillips 1975) and witches' broom formation in dwarf mistletoe infections.

In contrast to the inhibitory effects of ABA, promotive effects of ABA were observed with assimilate sinks (Schussler et al. 1984). Higher concentrations of ABA are associated with assimilate accumulation in seeds and storage tissue. This is consistent with the observations of another parasitic plant, dodder. As in the dwarf mistletoes, dodder is capable of diverting assimilates from other parts of the host to the site of infection (Tsivion 1978, Wolswinkel 1974a). Different portions of the host and *Cuscuta* were assayed for growth inhibitors to wheat seedlings (Ihl and Jacob 1980). The fractions which showed growth inhibition contained ABA. The highest concentration of growth inhibitor was in the haustoria of the dodder, i.e. the site where assimilates are transferred from the host to the parasite. This corresponds with transfer of assimilates into the seed or storage organs where elevated concentrations of ABA are found (Schussler et al. 1984).

ABA concentrations may vary according to the type of nutrient sink. Nutrient sinks associated with active growth of plant tissue and metabolism of assimilates have reduced concentrations of ABA (e.g. buds initiating growth, Dumbroff et al. 1979) while sinks associated with the accumulation of assimilates have high concentrations of ABA (e.g. seeds, Schussler et al. 1984). The situation with dodder may indicate a characteristic that distinguishes the two types of sinks. At the host-parasite interface, nutrients must move across the apoplast from the host phloem cell (Dörr 1972, Wolswinkel 1974b) from apparently a concentration of low ABA to high ABA (Ihl and Jacob 1980). It is not known if a similar ABA gradient occurs across the apoplastic transfer region in seeds or other storage organs. However, no apoplastic transfer is required for assimilates to enter a region actively growing such as elongating buds and young leaves. Here assimilates enter a region of lower ABA concentration, a condition which may be due to the rapid metabolism of ABA in these actively growing regions (Everat-Bourboulous 1982). We speculate that the symplastic transport of assimilates is directed to lower



concentrations of ABA, but apoplastic transport of assimilates is directed to higher concentrations of ABA. Associating ABA concentrations with variation in transport of assimilates does not imply cause and effect relationships. Whether ABA actually controls assimilate partitioning is not known.

Dwarf mistletoe infections would involve both symplastic and apoplastic transport of assimilates. Dwarf mistletoe infection of conifers stimulates growth resulting in branch swellings and broom formation. Such changes in growth were associated with the lower levels of ABA and increase in the symplastic transfer of assimilates. It would be interesting to investigate the possibility that low levels of ABA in this tissue were due to ABA metabolism. Dwarf mistletoe also has the apoplastic transfer of assimilates between host tissue and endophytic system of the parasite (Alosi 1978, Alosi & Calvin, these proceedings). We hypothesize that the ABA concentrations may be higher in the parasite cells than in the host cells at this interface. Such measurements were not practicle in this study. A test of the hypothesis that ABA is involved in regulating the directed transport of assimilates would be to test if exogenous applications of ABA could disrupt the transport of assimilates from the host to the dwarf mistletoe.

Changes in ABA concentrations in branch segments analyzed in this study are consistent with the hypothesis that symplastic transport of nutrients is directed to lower concentrations of ABA. Noninfected branches (sources of assimilates) show higher concentrations of ABA in one yr old branch segments than three yr old branch segments; the assumed export of assimilate coincides with a decrease in ABA concentrations. In the infected branch where no export of assimilates occurs, no difference in ABA concentration is seen between the two ages of branch segments. The differences in ABA concentrations between the two types of branch segments in the noninfected tissue do not exist in April (before bud break) and October (after dormancy), times of the year when export of assimilates would not be expected.

**Additional Considerations.**--Mechanisms by which plant growth substances affect dwarf mistletoe infections are complex. In addition to the growth substances already mentioned, gibberellins also are associated with diseases causing abnormal excesses in plant growth (Sequeira 1973) and could be involved in growth changes associated with dwarf mistletoe infections, but such measurements have not been made. Alterations in presence and relative amounts of plant growth substances due to dwarf mistletoe infection will have different impacts on the host. Some effects will be localized (e.g. increase cell division, membrane changes) while others will have more distal effects on the host. Cytokinins have been suggested to regulate one of these distal effects, i.e. directing assimilates from noninfected portions to the infected areas. Data in this paper indicate ABA also could be

involved in assimilate partitioning. Future studies on dwarf mistletoe and plant growth substances should not be restricted to one compound. A good indication on how alterations in plant growth substances will impact tree growth would be to compare the alterations in the various plant growth substances in localized infections of dwarf mistletoe to the alterations of concentrations in systemic infections. The latter infection causes more pronounced changes in host growth.

It is reasonable to expect that several plant growth substances are involved in successful dwarf mistletoe infections, but to understand how these substances influence dwarf mistletoe infections will not be easily attained.

## CONCLUSIONS

Dwarf mistletoe infections alter the concentrations of cytokinins, IAA, and ABA in the stem tissue of conifers. There are increases in the concentrations of cytokinins and IAA in conjunction with decreases in ABA concentrations. With what is known about these plant growth substances, such alterations should be associated with sink formation, stem swelling, and loss of apical dominance of the host tissue resulting from dwarf mistletoe infection. Evidence from this study supports the hypothesis that alterations in plant growth substances are involved in successful infections of dwarf mistletoe.

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# Water Metabolism and Seedling Photosynthesis in Dwarf Mistletoes<sup>1</sup>

Richard D. Tocher, Steven W. Gustafson, and Donald M. Knutson<sup>2</sup>

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Abstract--Diurnal measurement of host and dwarf Mistletoe transpiration shows that the parasite transpires day and night and at several to many times the host's rate. Chlorophyll content of dwarf mistletoe seeds is low. Still, seedlings consume less O<sub>2</sub> in light than in dark and light fixation of CO<sub>2</sub> probably provides important support for survival.

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## INTRODUCTION

Mistletoes represent a major stress on their hosts with respect to water loss. In most of the literature to be discussed, the mistletoe out-transpired its host on a surface area or tissue mass basis by a wide margin. Meanwhile, the host usually retained control of its own transpiration by the usual mechanism of stomatal closure during drought or at night.

Perhaps the most vulnerable stage in the life cycle of dwarf mistletoe is the eight to nine month period from expulsion of the fruit from the mother plant to the time when penetration of the new host site occurs. There has been some speculation on the photosynthetic capacity of the seed and resulting seedling based on the visual presence of chlorophyll but only one very brief report of the actual measurement of photosynthetic activity has appeared (Muir 1975). Further studies to quantify chlorophyll content and photosynthetic activity of the fruits and seedlings are obviously needed.

## WATER METABOLISM

### Literature Review

Recognition came early that mistletoes were a major water drain on their hosts. Kamerling in 1914 used the "detach and weight" method to estimate water loss in *Viscum* and some tropical members of the Loranaceae (1914a,b). In another report (1914c) he showed that tropical Loranths lost up to 50% more water than their hosts on a surface area basis. The difference in rates

between host and parasite was most dramatic during the hottest part of the day.

A series of studies by Harris and his co-workers from 1916-1934 consisted of measurement of osmotic potential by freezing point depression. They found that expressed sap from the parasite generally had a lower osmotic potential than the sap from the host, except under unusually favorable soil moisture conditions.

Wood (1924) studied *Loranthus* sp. parasitizing *Acacia* in arid regions of Australia. The parasite was lavish in its transpiration, greatly exceeding the host's transpiration.

Korstian (1924) found that osmotic concentrations in three species of *Arceuthobium* and one *Phoradendron* were generally higher than the osmotic concentrations of their hosts.

Vareschi and Pannier (1953) found that two tropical *Phoradendron* spp. did not restrict their transpiration during the dry season, and they reported rates as high as 1.2 g H<sub>2</sub>O dm<sup>-2</sup> h<sup>-1</sup> for the parasites in both wet and dry seasons.

*Struthanthus* sp., a tropical loranth, transpired even when its host, *Erythrina* sp., was leafless during the dry season (Marques-Valio et al. 1962). The leaf water content of the parasite was as high in the mornings in the dry season, as it was in the wet season on a surface basis (DeOliveira et al. 1962). The host possessed better control of water loss, and could restrict it more quickly than the parasite. The host had more stomates per surface area than the parasite.

Mark and Reid (1971) measured xylem water potentials of *Pinus contorta* and its parasite *Arceuthobium americanum* in Colorado. Water potential gradients favored the mistletoe obtaining water from the host even when the latter was under considerable water stress. The average water potential of the pine was -14.7 bars, while the average for the mistletoe was -21.3 bars.

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Hellmuth (1971a,b) compared  $\text{CO}_2$  fixation and transpiration of the mistletoe *Amyema* sp. parasitic on *Acacia* sp. Net photosynthesis was 50% higher in the parasite than the host, while transpiration during times of water stress was three times the rate of the host. Under moisture sufficient conditions the host slightly out-transpired the mistletoe.

Fisher (1975,1983) carried out extensive experiments on *Arceuthobium-Pinus* water relationships. He used thermocouple psychrometers to monitor the air stream before and after passing through cuvettes enclosing the transpiring tissues. He found that mistletoe transpiration rates were up to four times the rate of the host on a surface area basis. In other experiments, pine seedlings were grown in solution cultures containing varying amounts of polyethylene glycol 4000 as an osmoticum to obtain osmotic potentials ranging from 0 to -15 bars. The *Arceuthobium* spp. generally showed a lower water potential than the host, varying from -3 bars difference with a well watered host to -6 bars difference when the host was in the lowest potential culture solution.

#### Materials and Methods

Young conifers with mistletoe aerial shoots (*Pinus contorta* and *Arceuthobium americanum*; *P. ponderosa* and *A. campylopodum*; *Tsuga heterophylla* and *A. tsugense*) were grown in peat pots. The surface area of a needle tuft of host pine was measured by counting the needles and measuring the lengths of all needles in the clump and the average diameter of the needle fascicles. The surface area of needles was the outer surface area of the fascicle cylinder plus the flat rectangular areas on the inside of the fascicle. In these pines, stomates occur on all surfaces in approximately equal number per unit area.

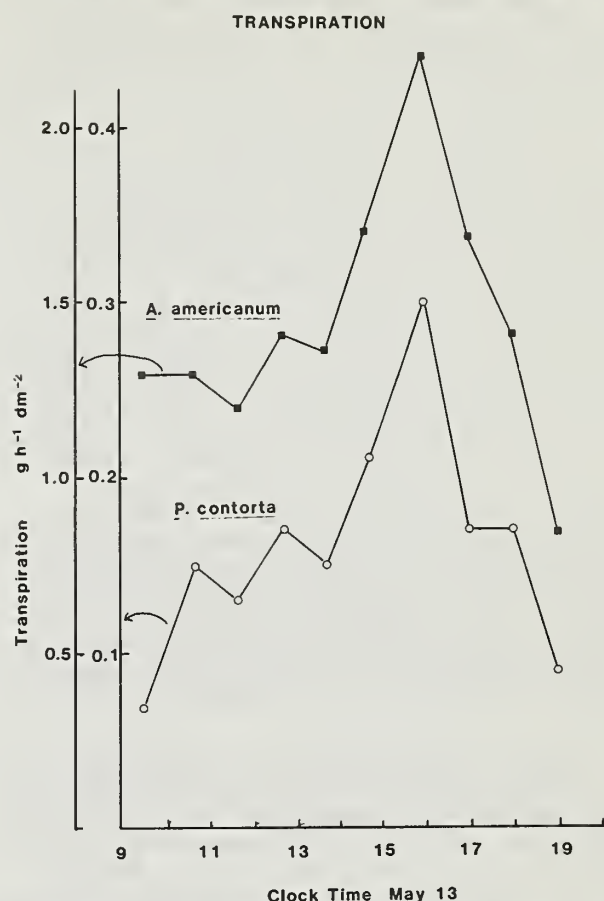
In *Tsuga heterophylla* the number of needles on a branch was counted, lengths were measured and the average needle width determined. The more or less rectangular hemlock needles bear stomates only on the lower surface, so the upper surface was not included in the surface area. A clear sleeve of polyethylene was placed around the parasite aerial shoots or host needles. A piece of rubber tubing was sealed in each open end with plasticene and light string. One tube was open to the air, the tube from the other end of the plastic sleeve led to a dry ice-ethanol trap and a drierite trap and then to a suction pump. Three lines of tubing were used: one from a sleeve enveloping host needles, one from a sleeve around a collection of attached aerial shoots and one pulled ambient air through a pair of traps to provide a correction for the moisture content of the air.

#### Results

##### Lodgepole Pine

Figure 1 presents a time course of transpiration of *Arceuthobium americanum* growing on *Pinus*

FIG. 1. COMPARISON OF PINE AND DWARF MISTLETOE



*contorta*. The rates are expressed as grams of water transpired per square decimeter per hour. The host was well watered. In order to present the data compactly on one graph, two vertical axes are shown, the left one spanning 0-2 g shows rates of the mistletoe transpiration, while the inner vertical axis spanning 0-0.4 g per hour shows the rates of lodgepole pine transpiration. In the early morning the mistletoe transpiration was about 18 times the host rate. Both peak at the same time, 4 PM daylight time, when the mistletoe rate was about seven times that of the host. In this second experiment (fig. 2) the same two vertical axes are used. The host had not been watered for seven days and the pine's transpiration rate at 1:30 PM, just before a thorough watering, was only one-fortieth that of the mistletoe. Over the next two hours the host transpiration recovered to about one-fifteenth the rate of the mistletoe.

##### Western hemlock

In this experiment the rates of transpiration of western hemlock are compared with *Arceuthobium tsugense* (fig. 3). Since the rates were more comparable than in the pine experiments just shown, the data are referred to only one vertical axis. The host had sufficient water in this experiment. Their rates were approximately equal early in the morning, but by 10 AM the mistletoe

rate exceeded the rate of the host and continued to rise more sharply until early afternoon when the mistletoe transpiration was about 2.3 times the rate of the hemlock. At only one reading (8 AM) did the host transpiration exceed that of the mistletoe.

In another experiment with hemlock (fig. 4) water had been withheld for six days and the hemlock before watering was transpiring only one-tenth to one-half as much as in figure 3 while the mistletoe was actually transpiring 2-3 times more than when water was sufficient. Note that we have two axes here; the inner left hand axis gives hemlock rates, while the mistletoe rates refer to the outer left hand axis. After watering the host's transpiration continued to fall for about an hour before it recovered, and at peak time, about 3 PM daylight time, its rate was only one-half as much as it had been in figure 3. After sunset host transpiration fell to zero and remained there during the night. The mistletoe on the other hand continued to transpire at 2-2.5  $\text{g dm}^{-2} \text{h}^{-1}$  during the night. The time plotted was the midpoint of the interval, thus the reading shown as 1:15 AM spanned the interval from 11 PM - 03:30 AM. Unfortunately the dwarf mistletoe reading at 10 PM was lost, as excess ice plugged the tube in the dry ice trap stopping the flow of air.

Table I shows variation of transpiration rates with time, and xylem potential measured with a PMS manufactured pressure bomb. The three right hand columns give the transpiration rates of the mistletoe, the host and their ratios, and represent the same data points as the previous time course. Note that at 10 AM, just before watering,

FIG. 2 WATER STRESS AND RECOVERY OF PINE

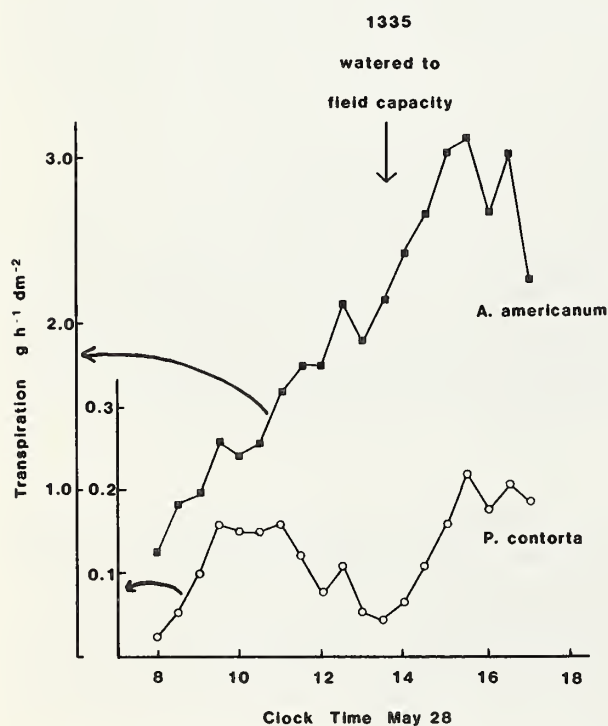
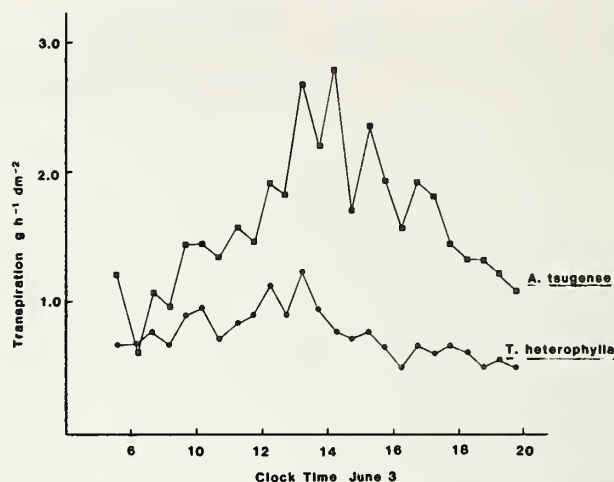


FIG. 3 COMPARISON OF HEMLOCK AND DWARF MISTLETOE TRANSPIRATION



the parasite's transpiration rate was 62 times that of the host!

The xylem pressure potentials were measured several times during the experiment, using freshly detached hemlock twigs or mistletoe aerial shoots from comparable heights on the tree. The paucity of suitable aerial shoots limited the number of samples. During the day the mistletoe had slightly lower pressure potentials but those became strikingly lower at night. The pressure bomb gauge upper limit was 40 bars. The first sample blew out of the chamber as the gauge passed -40 bars. A second sample remained in place, but no moisture had appeared on the cut stem at -40 bars.

#### Ponderosa pine

The results of a 24 h experiment using a 1.3 m. Ponderosa pine in a 10 gallon peat pot are given in figure 5. The axis values for *Arceuthobium campylopodum* (extreme left axis) are ten times the values for the pine. The pine at this time was probably water stressed. Nighttime transpiration was substantial in both plants--one-third of the peak rate in the day for the pine, and one-half the peak day rate at night for the mistletoe. Just before watering the pot the second morning, a small soil sample was taken from the surface soil in the pot, weighted, oven dried and

Table 1.--Variation of pressure potential and transpiration rate with time.<sup>1</sup>

Time	Xylem pressure potential		Transpiration rate		
	Mistletoe	Hemlock	Mistletoe	Hemlock	Ratio
	----- bars -----		--- $\text{g h}^{-1} \text{dm}^{-2}$ -----		
0800	--	--	2.8	0.14	20
1000	--	--	2.6	0.042	62
1020	Watered host to field capacity				
1040	-16	-13.5	3.4	0.34	10
1315	-22.5	-13.5	4.2	0.51	8
1515	-17.5	-12.5	4.6	0.57	8
1730	--	--	4.3	0.51	8
1930	-21.5	-8.5	2.2	0.091	24
0330	-40	-6.5	2.4	0.00	

<sup>1</sup>Western hemlock, 0.6 m tall in a 5 gal peat pot; water had been withheld for six days prior to experiment. Transpiration data as in figure 4.



wetted to field capacity. At the time the sample was taken, water content in the soil was 4.9%, or 13% of the soil's field capacity (37% of oven dried weight). This low level of soil moisture did not appear to inconvenience the pine very much as the transpiration rate was increasing before the watering.

## SEEDLING PHOTOSYNTHESIS

### Literature Review

Most dwarf mistletoes ripen and expel their single seeded fruits from August to October. When they strike a host they cement themselves to it with a hygroscopic pectic material called viscin. Germination occurs in the spring and parasitic contact usually occurs a few weeks later in early summer. Thus, the fruit and subsequent seedling survives up to nine months on its own stored food and photosynthetic capacity. There is some doubt on the period of dormancy required. Wicker (1974) reported that four species of *Arceuthobium* did not germinate immediately after dispersal, but required only about two weeks of cold storage to yield 55-80% germination. In apparent contrast to this, Beckman and Roth (1964) and Knutson (1974) found maximum germination of 50-80% only after 80-180 days of cold storage.

There have been several observations that dwarf mistletoe seeds contain chlorophyll and this has resulted in speculation that the seeds have

photosynthetic capability. Cohen (1963, in a description of the anatomy of the seedling, described the stomates, attributing them with the function of gas exchange during "probably photosynthesis in the green, long-lived seedlings." Kuijt (1969) referred to the chlorophyllous endosperms of the Viscaceae as "seats of great photosynthetic activity." Scharpf (1970) commented on the possibility that photosynthesis occurs in the chlorophyllous seeds, supplying energy necessary for establishment on the host. Muir (1975) did two experiments in which he exposed ten germinated seeds each to  $^{14}\text{CO}_2$  in light and in dark. He reported that the seeds exposed for 48 hours in light averaged 2,440 cpm and in dark averaged 310 cpm. He concluded that although the amount of  $\text{CO}_2$  assimilated was small, it may play a significant role in the supply of energy to the seeds.

### Materials and Methods

Chlorophyll was extracted by blending fruits or freeze-dried aerial shoots in 80% acetone for 15 min. with the blender container partly immersed in a cold water bath. Absorbance of an appropriate dilution of the extract was measured in a spectrophotometer and the concentrations determined using the formulæ from Holden (1965).

Respiration and photosynthesis by seedlings were measured by three methods: (1) with a Warburg respirometer with the flasks lighted ( $8.6 \times 10^4$  lux) or darkened with the flasks being

FIG. 4 WATER STRESS AND RECOVERY OF HEMLOCK

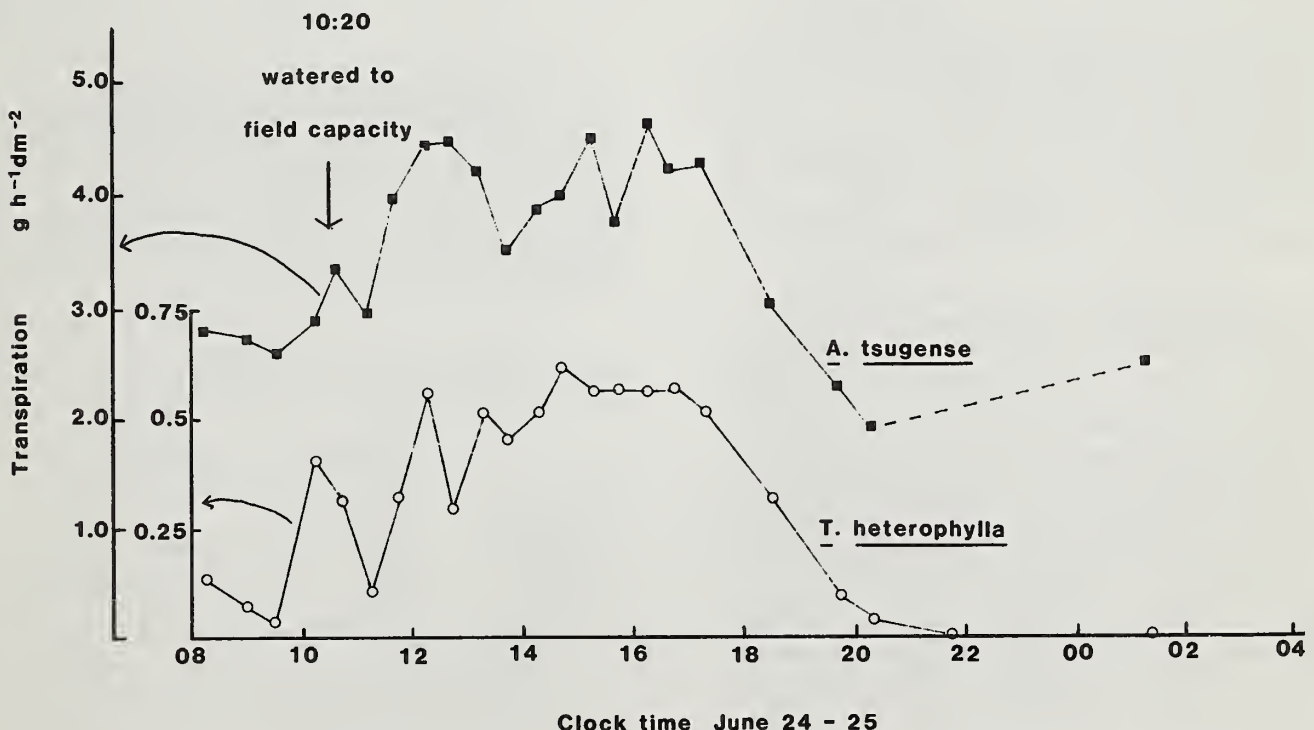
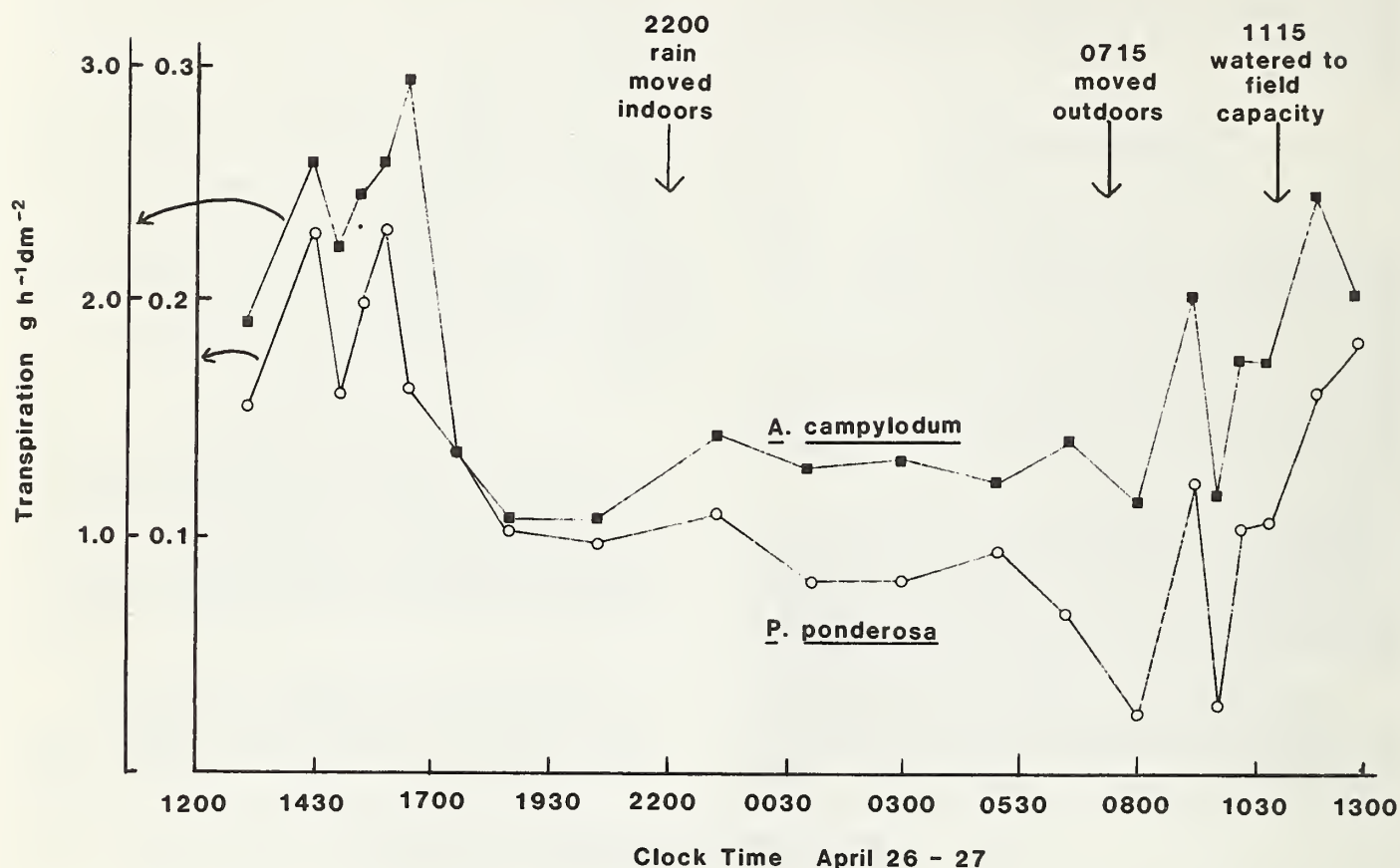


FIG.5 COMPARISON OF PONDEROSA PINE AND DWARF MISTLETOE TRANSPIRATION



wrapped with aluminum foil. The  $\text{CO}_2$  level was maintained at 1% with Pardee buffer (Umbreit et al. 1959). (2) polarographically using an oxygen electrode with the seeds immersed in an  $\text{O}_2$  saturated solution. Light intensity was  $1.3 \times 10^4$  lux, or darkness was maintained by wrapping the chamber with heavy cloth. (3) by measuring  $^{14}\text{CO}_2$  uptake during 24 h in darkness or light ( $8.6 \times 10^4$  lux). The distribution of label was measured by the protocol in figure 6.

**Seed germination.**--Seeds were collected in the autumns of 1975-77 and stored on filter papers suspended in a closed jar over saturated sodium chloride solution to maintain 75% relative humidity. The jar was kept at  $4^\circ\text{C}$ . Two to four months of stratification was given. As needed, seeds were soaked one hour in 2 or 3%  $\text{H}_2\text{O}_2$ , rinsed with sterile distilled water and germinated in petri dishes watered with sterile water. Chlorine bleach diluted to 1-5% reduced germination sharply, but failure to surface sterilize the seeds invariably caused them to be killed by rampant fungus infection. Germination rates were always lower than others have reported--our best rate with the 1976 seed lot was 53%, 1975 seeds gave 30% and 1977 seeds about 12% germination.

## Results

The chlorophyll contents of seeds and aerial shoots of the four species analyzed is shown in Table II. Hull and Leonard (1964) reported aerial shoot chlorophyll levels of 0.24-0.48 mg/g tissue for two species of *Arceuthobium*, an amount which represented 12 to 24% of the chlorophyll levels of their hosts. As we will show, the low level of chlorophyll correlates rather well with a low rate of photosynthesis by the seeds.

Table 2.--Chlorophyll content of dwarf mistletoes.

	Seeds <sup>1</sup>		Aerial Shoots <sup>1</sup>	
	chl a+b mg g <sup>-1</sup> FW	a/b ratio	chl a+b mg g <sup>-1</sup> FW	a/b ratio
<i>A. americanum</i>	0.31	1.38	0.35	1.92
<i>A. campylodum</i>	0.34	1.43	0.35	1.50
<i>A. douglasii</i>	0.39	1.35	0.38	2.80
<i>A. tsugense</i>	0.25	1.27	0.31	1.82

<sup>1</sup>Each figure is the mean of three or four extracts.



**Respiration and Photosynthesis.**--These measures were carried out with *A. campylopodium* seedlings which had been started ten days before. Visible germination (radical emergence) occurred three days before. Table III shows the results of three experiments with *A. campylopodium* seedlings, 10 in each Warburg flask. The mean value for O<sub>2</sub> uptake in the dark was 352  $\mu$ l per gram, and 204  $\mu$ l per gram in the light. We assume that the respiratory quotient (R.Q.) and photosynthetic quotient (P.Q.) are both equal to one, that is that equimolar amounts of O<sub>2</sub> and CO<sub>2</sub> are involved in both dark and light measurements. Subtracting the light rate from the dark rate gives a value of 148  $\mu$ l O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> as the apparent photosynthesis rate, which is equivalent to 42% of the O<sub>2</sub> consumed in dark respiration. Miller and Tocher (1975) reported that aerial shoots of *A. tsugense* showed apparent photosynthesis that was 20-34% of the rate of respiration. Some of this difference might be ascribed to the 10% greater chlorophyll content of *A. campylopodium* seeds as compared with *A. tsugense* aerial shoots.

Polarographic measurements of light and dark O<sub>2</sub> consumption gave closely similar values to the ones we obtained with the Warburg apparatus, 353  $\mu$ l O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> in the dark and 214  $\mu$ l O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> in the light. These figures were the averages of four experiments each.

A respiration rate of 350  $\mu$ l g<sup>-1</sup> h<sup>-1</sup> is equivalent to 0.688 mg CO<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> if the R.Q. is 1.0. If the respiration rate is constant during 24 hours, each seedling loses 48  $\mu$ g CO<sub>2</sub> or 1.6% of its fresh weight per day. In a 12 h light period each seedling can fix 10  $\mu$ g CO<sub>2</sub> per day or 21% of the amount lost in 24 h. Photosynthetic carbon fixation may provide the difference between life and death during the few weeks after germination before a food supply from the host can be obtained.

**<sup>14</sup>CO<sub>2</sub> Incorporation by *A. Campylopodium* Seedling.**--The results from <sup>14</sup>CO<sub>2</sub> labelling of seeds in light and dark and subsequent analysis of location of <sup>14</sup>C are reported in Table IV and V. The results show that seeds of *A. campylopodium* assimilated CO<sub>2</sub> in light. CO<sub>2</sub> assimilation in the light was approximately 350 times CO<sub>2</sub> assimilation in the dark. The seeds in light incorporated 25% of the original <sup>14</sup>CO<sub>2</sub> present in the reaction flask during 24 hours. However, it should be remembered that the seeds are respiring at a greater rate than they are photosynthesizing, thus in the course of twenty-four hours substantial

Table 3.--Oxygen uptake by *A. campylopodium* seedlings in light and dark.

Experiment Date	Manometric oxygen uptake <sup>1</sup>	
	Dark ( $\pm$ 1 S.D.)	Light ( $\pm$ 1 S.D.)
Feb. 3, 1977	369 $\pm$ 42	240 $\pm$ 6
Jan. 19, 1978	357 $\pm$ 40	108 $\pm$ 13
Mar. 23, 1978	330 $\pm$ 17	266 $\pm$ 46
Mean	352 $\pm$ 20	204 $\pm$ 85

<sup>1</sup> Data from three to six flasks, each containing 10 seedlings. microliters of O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>.

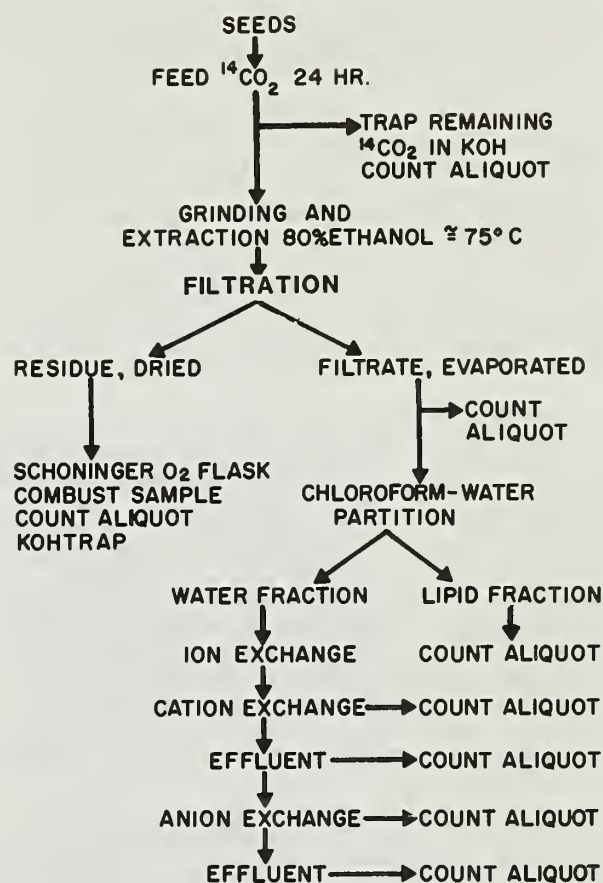


Figure 6. Flow chart for <sup>14</sup>C Label Procedure and analysis

Table 4.--<sup>14</sup>C label distribution in *A. campylopodium* seedlings.

Fraction	Dark fixation <sup>1</sup>	Light fixation <sup>2</sup>
	dpm	dpm in thousands
Total <sup>14</sup> C incorporated	560	342
Solid residue	560	6.5
Ethanol soluble	0	336
Chloroform soluble	0	28
Water soluble	0	214
Not accounted for	0	94

<sup>1</sup>Mean of two runs

<sup>2</sup>Mean of four runs

Table 5.--<sup>14</sup>C Distribution by ion exchange chromatography.

Sample	Cationic	Anionic	Neutral
----- % of water soluble -----			
1	11	29	60
2	25	22	53
3	24	21	55
4	17	15	67
Mean	19	22	59

dilution of the labelled  $^{14}\text{CO}_2$  took place. Therefore, quantification of photosynthetic rates of the seeds by this method is not reliable.

Extraction of seeds with ethanol showed that 97% of the incorporated  $^{14}\text{C}$  was ethanol soluble, of this, 83-90% of the activity in the ethanol extracts concentrate was  $\text{H}_2\text{O}$  soluble. The  $\text{H}_2\text{O}$  soluble phase was separated into cationic, anionic and neutral fractions by ion exchange chromatography. These results are reported in Table V. The majority (50-67%) of the label remained in the neutral fraction, while 11-25% of the label was in the cationic fraction, and 15 to 29% of the label was in the anionic fraction.

Miller and Tocher (1975) reported the following values from ion exchange chromatography of water soluble  $^{14}\text{C}$  from aerial shoots: cationic fraction, 12-26%; anionic fraction, 20-25%; and neutral fraction, 51-68%. Hull and Leonard (1964) reported a smaller percentage (one percent) of label in the cationic fraction, and a larger percentage (36-70%) in the anionic fraction. They suggested the presence of Crassulacean acid metabolism (CAM) or of enzymes active in CAM in aerial shoots based on the large amounts of  $^{14}\text{C}$  label incorporated into malic acid. The non-existant to very minute assimilation of  $^{14}\text{C}$  by the seeds of *A. campylopodum* in the dark suggests the absence of CAM.

Tainter (1971) suggests that chloroplasts of *A. pusillum* resembled chloroplasts associated with  $\text{C}_4$  carbon assimilation systems. The  $\text{C}_4$  pathway is usually associated with a certain type of cell anatomy within leaves. Mesophyll cells have chloroplasts which fix  $\text{CO}_2$  by PEP carboxylase to form malate. The malate is either directly transferred or is aminated to form aspartate and then transferred to the bundle sheath cells. In the bundle sheath cells the  $\text{CO}_2$  carrier is decarboxylated. The  $\text{CO}_2$  is then fixed in the chloroplast via the Calvin cycle (Bonner and Varner 1976). This elaborate anatomy has not been reported to occur in dwarf mistletoes. However, that does not exclude the possibility of the presence of  $\text{C}_4$  system enzymes. It is apparent that further investigation of the pathway of carbon fixation in the seed is needed.

#### CONCLUSIONS

Water Metabolism.--Thus, we can say that the tree dwarf mistletoes studied, *A. americanum*, *A. campylopodum* and *A. tsugense* out-transpire their hosts under almost all conditions of soil moisture stress or darkness. Moreover the transpiration rates observed were usually several to many times the rates of the hosts, particularly when host stress was greatest. The only exception was *A. tsugense* when its host was well supplied with water.

Seedling Photosynthesis.--Seeds of *a. campylopodum* were quite vulnerable to fungal attack during incubation in the laboratory. Presoaking of seeds with two and three percent

$\text{H}_2\text{O}_2$  resulted in less fungal contamination and in relatively high germination percentages as compared to results obtained using Chlorox pretreatments. The seeds were found to contain chlorophyll in concentrations (0.25 to 0.40 mg/g fresh weight) comparable to those in aerial shoots which are ten to twenty percent the amounts found in host leaves (Hull and Leonard 1964). The variance in chlorophyll concentrations in seeds and aerial shoots of different species supports the hypothesis of Miller and Tocher (1975) that different species of *Arceuthobium* vary in their dependence upon the host.

$\text{O}_2$  consumption by seeds in light and in dark was measured by manometric and polarographic methods. The resulting data showed good agreement. The difference between the light and dark rates was attributed to  $\text{O}_2$  evolved during photosynthesis. This mean difference equaled  $148 \mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . This figure equaled 42% of the dark respiration rate of  $352 \mu\text{l O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . The  $^{14}\text{C}$  labelling experiments proved that the seeds fix  $\text{CO}_2$  in the light. This  $\text{CO}_2$  fixation in light supports the previous reasoning that the difference between  $\text{O}_2$  uptake in the light and in the dark was attributed to  $\text{O}_2$  evolved in the light reactions of photosynthesis. Further investigation of the pathway of  $\text{CO}_2$  fixation in seeds and shoots of dwarf mistletoe is warranted.

This study supports the earlier hypothesis of Cohen (1964), Kuijt (1969), and Scharpf (1970), that the chlorophyll present in dwarf mistletoe seeds is functional. However, Kuijt (1969) appears to have been overly enthusiastic in his description of Viscacean seeds as "seats of great photosynthetic activity."

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# Host Resistance to Dwarf Mistletoes<sup>1</sup>

Robert F. Scharpf<sup>2</sup>

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Abstract.--Resistance to dwarf mistletoes (*Arceuthobium*) is expressed mainly by varying degrees of infection among host species. Some resistance occurs within certain dwarf mistletoe-host species combinations, however. Such resistance appears to result from both morphological and physiological characteristics of the hosts.

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Resistance of certain coniferous tree species to infection by dwarf mistletoes (*Arceuthobium*) has been recognized for nearly as long as investigators have been observing and studying these parasites in the field. Many dwarf mistletoes have definite host preferences. And yet infection occasionally is found on tree species other than the primary host. One of the first taxonomic systems of classification was based on host relationships without regard for "biological parity" (Gill 1935). A later taxonomic classification of the taxa is based on morphological characteristics (Hawksworth and Wiens 1972). In spite of the morphological integrity of most dwarf mistletoes, Hawksworth and Wiens (1972) recognize definite host preferences by the various species and subspecies and use them to help identify the taxa. They devised a "susceptibility class" system based on the percentage of trees of a species that are infected within 20 feet of an infected "main" or "principal" host (table 1). This classification system rates the relative susceptibility (resistance) of hosts to dwarf mistletoe species in the forest.

In nature, about two-thirds of the dwarf mistletoes parasitize species other than their principal hosts. Infection of these other hosts is highly variable and often difficult to explain. A species like *A. laricis* in the western United States, for example, occurs on members of at least four other genera in the Pinaceae, whereas *A. apachecum* and *A. blumeri* are found only on *Pinus strobiformis* (southwestern white pine). Strange host relationships that defy the imagination also have been observed (table 2). For instance, in mixed conifer stands in California, *A. abietinum* f. sp. *concoloris* growing on *Abies concolor* (white fir) cannot infect *A. magnifica* (red fir), and *A.*

*abietinum* f. sp. *magnificae* growing on red fir cannot infect white fir in stands where abundant opportunity exists for them to do so (Parmeter and Scharpf 1963). But, *A. abietinum* f. sp. *concoloris* can occasionally infect *Pinus lambertiana* (sugar pine), *P. contorta* (lodgepole pine), and *P. monticola* (western white pine). In addition, when these unusual hosts are infected, more than one infection often occurs. These strange crosses often result in an "all or nothing" mode of parasitism of the tree (Hawksworth and Wiens 1972). For example, *Picea engelmannii* (Engelmann spruce) is rarely parasitized by *A. americanum*, but when it is, it often bears 100 or more infections per tree (Hawksworth and Graham 1963). Why certain dwarf mistletoes cannot infect a closely related host species but can cause severe infection of trees of a different genus remains a mystery.

To further confound our understanding of resistance, large geographic areas of apparently susceptible, native host species are uninfected by dwarf mistletoes, whereas other areas are heavily infected. Absence of *A. vaginatum* on *P. ponderosa* (ponderosa pine) in the Black Hills area of South Dakota (Hawksworth 1963), and lack of *A. douglasii* on *Psuedotsuga menziesii* (Douglas-fir) along much of the Pacific coast (Wicker 1969) are examples of this phenomenon. Why these hosts are free of dwarf mistletoe in these locations and infected in others is not fully known, but certain climatic factors appear to play an important role in some cases.

The lack of resistance to dwarf mistletoe among some introduced, conifer species planted within the range of dwarf mistletoes in the field (table 3) is an equally perplexing problem. A striking example of this was a 55-year-old plantation of *P. sylvestris* (Scotch pine) that became heavily infected by larch dwarf mistletoe, *A. laricis* in Washington (Graham and Lephart 1961). In contrast, several native tree species that are immune or highly resistant to infection by dwarf mistletoes in the field have been infected by inoculation under artificial conditions (table 4). Why certain species are resistant in the field but susceptible under artificial conditions is not known. Perhaps certain climatic conditions or biotic factors in nature preclude infection.

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Table 1.--Five classes used to rate the relative susceptibility of hosts to dwarf mistletoes.

Host Class	Infection Factor	Remarks
I. Principal	At least 90 percent; usually nearly 100 percent	Uninfected trees are seldom found within the 20-foot zone unless they are small and stunted
II. Secondary	50 to 90 percent	--
III. Occasional (or tertiary)	5 to 50 percent	--
IV. Rare	More than 0 but less than 5 percent	--
V. Immune	0	Potential host trees not infected even where the dwarf mistletoe in question is common

Source: Hawksworth and Wiens (1972)

Table 2.--Reported incompatible host-parasite relationship.<sup>1</sup>

Arceuthobium	Host	Reference
<u>A. abietinum</u> f. sp. <u>concoloris</u>	<u>Pinus contorta</u> subsp. <u>murrayana</u>	Hawksworth & Wiens 1982
<u>A. abietinum</u> f. sp. <u>concoloris</u>	<u>Pinus lambertiana</u>	Hawksworth & Wiens 1972
<u>A. americanum</u>	<u>Picea pungens</u>	Hawksworth & Wiens 1972
<u>A. americanum</u>	<u>Pinus albicaulis</u>	Wier 1919b
<u>A. americanum</u>	<u>Pseudotsuga menziesii</u>	Hawksworth & Wiens 1972
<u>A. pusillum</u>	<u>Larix laricina</u>	Tainter & French 1967
<u>A. tsugense</u>	<u>Larix europaea</u>	Kuijt 1964
<u>A. tsugense</u>	<u>Larix occidentalis</u> <sup>2</sup>	Smith 1974
<u>A. tsugense</u>	<u>Picea engelmannii</u>	Hawksworth & Wiens 1972
<u>A. tsugense</u>	<u>Picea sitchensis</u>	Laurent 1966

<sup>1</sup> Updated from Hawksworth and Wiens (1972)

<sup>2</sup> Inoculations

In a few instances, infection of a host by one species of dwarf mistletoe seems to "exclude" or prevent infection by others (Hawksworth 1968; Hawksworth and Wiens 1972). For example, when P. contorta ssp. latifolia (lodgepole pine) is the principal host for A. americanum, A. cyanocarpum has

not been found; and in stands where lodgepole pine is the principal host for A. cyanocarpum, A. americanum does not occur (Hawksworth 1968). Another type of exclusion of infection by dwarf mistletoes has also been observed in some instances, but in this case members of the same species are

Table 3.--Reports of extra-limital hosts naturally infected by dwarf mistletoes.

Host	<u>Arceuthobium</u>	Locality	Reference
<u>Cupressus macrocarpa</u>	<u>A. oxycedri</u>	USSR	Zefirov 1955
<u>Juniperus thuifera</u>	<u>A. oxycedri</u>	USSR	Lazarev & Grigonov 1980
<u>Larix europaea</u>	<u>A. tsugense</u>	British Columbia	Kuijt 1964
<u>Picea abies</u>	<u>A. laricis</u>	Idaho	USDA 1963
<u>P. pungens</u>	<u>A. pusillum</u>	Maine	USDA 1960
<u>Pinus banksiana</u>	<u>A. laricis</u>	Idaho	Graham 1959a
<u>P. pinaster</u>	<u>A. campylopodum</u>	California	Kuijt 1960b
<u>P. halapensis</u>	<u>A. campylopodum</u>	California	Scharpf <sup>2</sup>
<u>P. resinosa</u>	<u>A. laricis</u>	Idaho	USDA 1962
<u>P. sylvestris</u>	<u>A. laricis</u>	Washington	Graham & Leaphart 1961
<u>P. sylvestris</u>	<u>A. americanum</u>	Washington	Graham & Leaphart 1961
<u>P. sylvestris</u>	<u>A. americanum</u>	Alberta	Powell 1968
<u>P. sylvestris</u>	<u>A. campylopodum</u>	Idaho	Weir <sup>3</sup>
<u>P. sylvestris</u>	<u>A. vaginatum</u>	Colorado	Hawksworth & Laut 1981

<sup>1</sup>Updated from Hawksworth and Wiens (1972)

<sup>2</sup>Unpublished data by Robert F. Scharpf

<sup>3</sup>Unpublished data by J. R. Weir

also excluded. Infected branches are usually highly resistant or immune from secondary infection by either the same or other dwarf mistletoe species (Hawksworth and Wiens 1972). The mechanisms by which dwarf mistletoes exclude one another are not known and is a fascinating area for further research.

In unusual host-parasite combinations, resistance is often expressed by certain growth responses of the mistletoe and morphological changes in the host. For instance, with infection of sugar pine by white fir dwarf mistletoe, shoots of the parasite develop slowly and are sparse, endophytic system growth appears slow, and excessive branch swelling occurs. Similar expressions of host-parasite incompatibility have been reported for other odd dwarf mistletoe-host combinations as well (Hawksworth and Wiens 1972).

#### PROBLEMS IN DETERMINING RESISTANCE

With a parasitic plant as widespread and as intensively observed and studied as the dwarf mistletoes, one would expect several clearcut examples of resistance of host species to a given dwarf mistletoe. But determining resistance from field observation alone can be a very questionable if not erroneous practice. As an example of the problem, one of the earliest tests of reportedly resistant ponderosa pines to A. vaginatum ssp. cryptopodum failed to show any differences between resistant and susceptible trees (Hawksworth and Edminster 1981). In these tests, several hundred progeny from "resistant" and "susceptible" trees were outplanted in 1932 for testing in a naturally

infested stand. A check of the surviving trees in 1959 showed that some were lightly to moderately infected and possibly resistant. However, another examination in 1979, showed the "resistant" trees did not differ significantly from "susceptible" trees in survival rate, percentage of trees infected, and average rated level of infection. In this case, it took nearly a half century of field testing to show that the initial field determinations of what constituted "resistant" trees were in error.

What are some of the problems in determining resistance of a host to a given dwarf mistletoe? The assumption that populations or geographic areas of trees that are free of infection are resistant to dwarf mistletoes is almost certain to lead to error. As previously discussed, large (and small) stands of susceptible hosts are often disease-free for unknown reasons. Climatic limitation of the parasite from portions of the host range has been suggested as a reason for this difference (Hawksworth 1956, 1963; Wicker 1969).

Geologic history is another factor that has regulated spread of dwarf mistletoe. For example, the absence of dwarf mistletoe in most of the stands around Sunset Crater, a volcano in Arizona that erupted about 900 years ago (Hawksworth 1960), illustrates how geologic history can confuse determinations of resistance based on field observations. In this case, the host was able to become reestablished on the site after the eruption more rapidly than the parasite was able to reinvade the new stands.

Because of the mechanism of spread of dwarf mistletoe, individual trees or groups of trees often escape infection, giving the appearance of



Table 4.--Extension of host ranges of Arceuthobium as determined by artificial inoculations on unnatural hosts.<sup>1</sup>

Host	Dwarf mistletoe	Reference
<u>Abies amabilis</u>	<u>A. laricis</u>	Smith & Wass 1972b
<u>A. concolor</u>	<u>A. campylopodum</u>	Weir 1918a
<u>A. grandis</u>	<u>A. campylopodum</u>	Weir 1918a
<u>Larix europaea</u>	<u>A. laricis</u>	Weir 1918a
<u>L. leptolepis</u>	<u>A. laricis</u>	Weir 1918a
<u>L. occidentalis</u>	<u>A. campylopodum</u>	Weir 1918a
<u>L. occidentalis</u>	<u>A. tsugense</u>	Smith 1970a
<u>Picea abies</u>	<u>A. campylopodum</u>	Weir 1918a
<u>P. abies</u>	<u>A. tsugense</u>	Smith 1965
<u>P. glauca</u>	<u>A. laricis</u>	Smith 1974
<u>P. glauca</u>	<u>A. tsugense</u>	Smith 1965
<u>P. engelmannii</u>	<u>A. tsugense</u>	Smith 1970b
<u>P. pungens</u>	<u>A. tsugense</u>	Hawksworth & Wiens 1972
<u>Pinus banksiana</u>	<u>A. occidentale</u>	Hedgecock & Hunt 1917
<u>P. bungeana</u>	<u>A. occidentale</u>	Hedgecock & Hunt 1917
<u>P. flexilis</u>	<u>A. apachecum</u>	Mathiasen 1978
<u>P. flexilis</u>	<u>A. blumeri</u>	Mathiasen 1978
<u>P. flexilis</u>	<u>A. microcarpum</u>	Mathiasen 1978
<u>P. mugo</u>	<u>A. campylopodum</u>	Weir 1918a
<u>P. palustris</u>	<u>A. occidentale</u>	Hedgecock & Hunt 1917
<u>P. pinea</u>	<u>A. occidentale</u>	Hedgecock & Hunt 1917
<u>P. ponderosa</u>	<u>A. tsugense</u>	Smith & Craig 1968
<u>P. radiata</u>	<u>A. tsugense</u>	Smith & Craig 1968
<u>P. resinosa</u>	<u>A. campylopodum</u>	Weir 1918a
<u>P. strobiformis</u>	<u>A. cyanocarpum</u>	Hawksworth & Wiens 1972
<u>P. strobis</u>	<u>A. cyanocarpum</u>	Hawksworth & Wiens 1972
<u>P. sylvestris</u>	<u>A. campylopodum</u>	Weir 1918a
<u>P. sylvestris</u>	<u>A. tsugense</u>	Smith & Craig 1968
<u>P. torrevana</u>	<u>A. occidentale</u>	Kuijt 1960a
<u>P. virginiana</u>	<u>A. occidentale</u>	Hedgecock & Hunt 1917
<u>Pseudotsuga menziesii</u>	<u>A. tsugense</u>	Smith & Wass 1972a
<u>Tsuga canadensis</u>	<u>A. tsugense</u>	Weir 1918a

<sup>1</sup>Updated from Hawksworth and Wiens (1972)

resistance. Adequate levels of inoculum (dwarf mistletoe seeds) and the presence of a suitable target are necessary for infection to occur (Wicker 1967, Wicker and Shaw 1967). In many cases, hosts are screened from seeds by non-host trees, wind patterns distribute seeds in unusual ways, and many variables can affect the inoculum before infection takes place.

In spite of these problems, several investigators have reported what they consider to be examples of resistance to dwarf mistletoes in the field. For example, in a study of infection of ponderosa pines by A. campylopodum in California, about 1 in 23 trees appeared to show some resistance to infection (Wagener 1965). In another study, one seed source of P. jeffreyi (Jeffrey pine) in an infected plantation showed noticeable resistance to infection (Scharpf and Parmeter 1967). Similarly, two instances were noted in which individual ponderosa pines in the southwest appeared resistant

to A. vaginatum ssp. cryptopodum (Hawksworth 1961). But the mechanism by which the trees in these cases resisted infection was not known, however. An interesting mechanism of resistance to A. campylopodum has been observed in ponderosa pine (Roth 1966). A "drooping" needle race of ponderosa pine is quite resistant because the seeds of dwarf mistletoe slide off the host rather than adhere and cause infection. In this case, a morphological characteristic of the host rather than a physiological response to infection imparts some level of resistance.

#### SELECTION AND TESTING FOR RESISTANCE

Relatively little effort has gone into selecting and testing for resistance to dwarf

Table 5.--Number of *A. campylopodum* plants developing on grafted ponderosa pines and on seedlings interplanted as controls. Progenitors of the grafts ("candidates") were selected for mistletoe resistance.

Test Clones	Number of trees	Mean Tree Height	Total Plants on all trees	Range among individual trees	Average plants per tree
		-cm-		number	
Resistant clone 1	13	62	0	0-0	0.0
Resistant clone 2	8	66	11	0-4	1.4
Resistant clone 3	5	66	1	0-1	0.2
Resistant clone 4	4	70	0	0-0	0.0
Resistant clone 5	5	88	18	0-10	3.6
Resistant clone 6	4	80	20	4-8	5.0
Resistant clone 7	3	51	1	0-1	0.3
Susceptible grafts	9	63	55	0-16	6.1
Deschutes nursery stock	27	84	433	6-43	16.0
Colorado nursery stock	10	77	134	2-32	13.4

Source: Roth (1974a)

<sup>1</sup>*P. ponderosa* var. *scopulorum*

mistletoes. Locating potentially resistant trees is a difficult task, and many managers believe that they can control damage from dwarf mistletoe with silvicultural methods (Roth 1974a).

A few deliberate efforts to find resistance to dwarf mistletoes have been undertaken. Hawksworth and Edminster (1981), failed to find any indication of resistance by ponderosa pine to *A. vaginatum* ssp. *cryptopodum* in a test begun in 1932. Some differences in resistance of ponderosa pine and Jeffrey pine to *A. campylopodum* have been shown in two more recent studies. In one, small potted ponderosa pines were grafted with "resistant" scions, placed in an infected stand, inoculated with seeds for 5 years, and examined for several years thereafter for infection (Roth 1974a). Results showed not only clear differences in resistance to infection among the test trees, but also differences in susceptibility to damage (table 5, figure 1). The mechanism of resistance in these tests was not determined, but only current years' tissues of ungrafted seedlings were regularly infected in contrast to less regular infection of shoots of more mature scions. Roth (1974a) suggested that anatomical features accompanying shoot differentiation are involved in resistance, but that physiological resistance cannot be overlooked. In a related study, ponderosa pine showed juvenile susceptibility to dwarf mistletoe (Roth 1974b). Scions from trees of various ages grafted to seedlings showed a decrease in frequency of infection with an increase in tree age. Susceptibility of trees of various ages has not been tested in the field, however. In contrast to Roth's findings on pine, I found small true firs in the field in California to be more resistant to infection than larger ones. In this study, tree size rather than age was more closely associated with infection. The mechanism by which small trees

resisted (or escaped) infection was not determined (Scharpf 1969).

The most recent tests to determine resistance of a host species to dwarf mistletoe are in progress in California. They are being conducted with a "resistant" seed source of Jeffrey pines (Scharpf and Parmeter 1967). In these tests, several approaches to determine resistance are being used. In the first approach, two plantations of Jeffrey pines planted nearly a half century ago adjacent to naturally infested overstory trees are being monitored about every 5 years for differences in susceptibility among various Jeffrey pine seed sources, starting in 1961. Resistance has continued

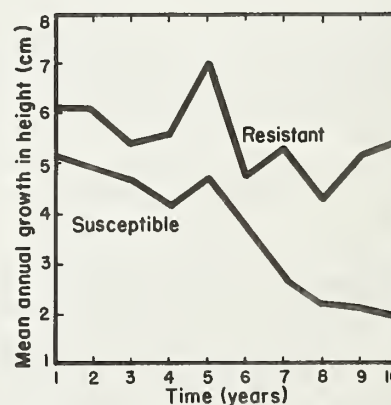


Figure 1.--Mean annual growth in height of clonal members from candidates 1, 3, 4 and 7, compared with susceptible controls and with nursery stock of "Deschutes" and "Colorado" seed sources (Roth 1974a).



to be found in one specific seed source. Periodic monitoring of these plantations will help determine if resistance persists over time.

The second approach to testing for resistance was begun in 1980 in another plantation of Jeffrey pines established with the same seed sources in the other study. These pines (7 years old in 1980) were ideal size for carefully controlled inoculation tests. In 1980 and 1981, about 15000 dwarf mistletoe seeds were placed on tree branches and followed for infection. Although results are preliminary at this time, the "resistant" seed source shows a level of resistance several times that of the more susceptible sources (table 6). Because at least one seed source of Jeffrey pine is consistently showing high levels of resistance, further research is underway to determine the mechanism(s) of resistance.

The third approach involves growing seedlings of the "resistant" Jeffrey pine and outplanting them along with the local seed source as controls, in naturally infested stands. One outplanting was established in Southern California in spring 1984, and more are planned for other areas in the future. With more research on mechanisms of infection, with the examination of existing field tests for several more years, and with outplanting of resistant and native seed sources in various areas, levels of resistance of several seed sources of Jeffrey pines should be established.

## CONCLUSIONS

Control of dwarf mistletoes is now most often accomplished by silvicultural methods of forest management. Treatments usually include removal of the infected species in a mixed species forest through thinning or harvest, or by clearcutting

Table 6.--Infections occurring on different seed sources of Jeffrey pine inoculated with A. campylopodum at Institute of Forest Genetics, Placerville, California, 1980-1981.

Jeffrey pine seed source (California)	Number of infections (9/83)	Percent of all infections
High elevation, east side Sierra Nevada (Alpine Co. 8000 ft)	106	57
Lake Tahoe (Eldorado Co. 5500 ft)	61	33
West side Sierra Nevada (Placer Co. 3500 ft)	18	10
Total	185	100

areas where heavy infection occurs in pure species stands. Unfortunately, not all stands can be clearcut or managed in ways to eliminate dwarf mistletoes or to avoid damage from these parasites. The availability of resistant planting stock would be an important addition to control options, particularly in cases where high values are at stake or where alternate tree species are not suitable or available.

Further studies are needed to identify resistant candidate trees or dwarf mistletoe resistant seed sources in the field. New methods or techniques need to be developed to "speed up" the process for screening and testing resistant selections. Further investigation is needed to better understand the mechanisms that impart resistance to trees. And, basic research is needed to provide information about the inherent characteristics of dwarf mistletoes and their hosts that will aid in selection and development of resistance.

With the continuing emphasis on intensive forest management and with increasing constraints on land use and management options, the development of dwarf mistletoe resistant conifers should be pursued as another approach to control these serious pathogens in Western North America.

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# Seed Development, Germination Behavior and Infection Characteristics of Several Species of *Arceuthobium*<sup>1</sup>

Donald M. Knutson<sup>2</sup>

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**Abstract.**--Seeds of *Arceuthobium* species account for 5-18% of mature fruit weight. Viscin accounts for 26-61% of mature seed weight. Repeated wet-dry cycles of mistletoe seeds reduce the water-holding capacity of viscin. Seed germination is improved with light and with time since harvest. Radicle elongation was 24-92 micrometers per day. *Arceuthobium* seeds are negatively phototropic, positively thigmotropic, and geotropically neutral. Greatest infection was at a low temperature-high light regime, but growth of both infected and uninfected trees was best at a high temperature regime. Growth of aerial shoots of *A. campylopodum* was best at the high temperature regime.

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## INTRODUCTION

Information reported here deals with observations on the basic biology of species of *Arceuthobium* native to Oregon. An original intent was to develop a body of information on the behavior of these species so they could be manipulated with some predictability. The plan was to develop greenhouse "model forests" of infected and uninfected seedlings which would be correlated with real-world stands and to develop management recommendations based on their responses to stresses and manipulations.

Before being forced to abandon this work, we developed a considerable body of data on the biology of *Arceuthobium* seeds. Since much of the work was not completed, we cannot draw statistical inferences from the data. However, the data obtained are biologically sound and were carefully collected. It is reported here in the hope that it will help develop a more complete understanding of the fascinating genus *Arceuthobium*.

The six Oregon dwarf mistletoes studied and their principal hosts are (1) *Arceuthobium*

*abietinum* Engelm. ex Munz f. sp. *concoloris* (hereafter abbreviated as *A. abietinum*) (on *Abies grandis* (Dougl. ex D. Don) Lindl.), (2) *A. americanum* Nutt. ex Engelm. (on *Pinus contorta* Dougl. ex Loud.), (3) *A. campylopodum* Engelm. (on *Pinus ponderosa* Dougl. ex Laws.), (4) *A. douglasii* Engelm. (on *Pseudotsuga menziesii* (Mirb.) Franco), (5) *A. laricis* (Piper) St. John (on *Larix occidentalis* Nutt.), and (6) *A. tsugense* (Rosendahl) G. N. Jones (on *Tsuga heterophylla* (Raf.) Sarg.). The research was conducted at the U.S. Forest Service Forestry Sciences Laboratory at Oregon State University, Corvallis.

The main topics to be discussed here are:

1. Fruit and seed development
2. Moisture content of seeds and viscin
3. Seed dormancy and germination
4. Radicle growth
5. Tropistic responses of germinated seeds
6. Influence of light and temperature on infection and subsequent growth of dwarf mistletoe and host trees.

## FRUIT AND SEED DEVELOPMENT

Throughout the decade of the 1970s, we determined the size and weights of fruits and seeds of 6 species of *Arceuthobium* native to Oregon. These measurements were taken in the second year of their maturation, from early June to late August. Selected results are shown in Tables 1 and 2.

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Table 1. Developmental increase in fruit and seed size of 6 species of Arceuthobium, 1978.  
Each datum based on 30 seeds.

Dwarf mistletoe	Fruit and seed length and width		
	6/14	7/20	7/20
	Fruit (mm)	Fruit (mm)	Seed (mm)
<u>A. abietinum</u>	2.7 x 1.5	4.5 x 2.1	2.4 x 1.2
<u>A. americanum</u>	-- --	4.6 x 2.4	2.0 x 0.9
<u>A. campylopodum</u>	4.0 x 2.0	4.9 x 2.4	2.1 x 1.1
<u>A. douglasii</u>	2.7 x 1.7	4.2 x 2.0	1.8 x 0.9
<u>A. laricis</u>	2.1 x 1.3	-- --	-- --
<u>A. tsugense</u>	-- --	4.0 x 1.7	-- --

Table 2. Developmental increase in fruit size and weight of 4 species of Arceuthobium, 1979.  
Each datum based on 30 seeds.

Dwarf mistletoe	Fruit length (mm) and weight (mg)					
	7/25		8/1		8/7	
	L	Wt	L	Wt	L	Wt
<u>A. abietinum</u>	3.9	11.8	3.5	7.3	3.7	9.7
<u>A. americanum</u>	4.0	13.7	4.1	14.3	4.2	15.0
<u>A. campylopodum</u>	3.7	10.5	4.6	24.8	5.0	30.0
<u>A. douglasii</u>	4.0	8.0	3.5	8.1	3.9	7.6

Because seeds are essentially undeveloped in June, the only measurements we could obtain in the early season were of fruit development. Measurements on seed development were not taken until late June.

Fruits showed a fairly uniform dimensional increase throughout the second growing season. Seed size increase followed a sharper gradient than that of fruit size. The rate of seed elongation for all 6 species increased dramatically between mid-July and mid-August. After mid-August, seed elongation nearly ceased, coinciding with a sharp increase in seed weight.

Fruits and seeds varied in size and weight from year to year. For example, seed and fruit weights of fruits and seeds collected in 1972 were smaller than those in 1971, and the seeds constituted a smaller percent of the total fruit weight.

Dwarf mistletoe	Seed wt. as % of fruit wt.	
	1971	1972
<u>A. abietinum</u>	16%	9%
<u>A. americanum</u>	18%	9%
<u>A. campylopodum</u>	14%	9%
<u>A. douglasii</u>	--	10%
<u>A. laricis</u>	--	5%
<u>A. tsugense</u>	16%	8%

In all years, however, seed size approximately doubled in a 30-day period between mid-July and mid-August.

Mature seeds of A. campylopodum were used to determine the relative weights of seeds and viscin. Viscin was removed from seeds with a teasing needle under a dissecting scope, and seeds and viscin were weighed separately. Viscin contributed 26-61% to the combined weight. (Each datum is based on 10 seeds).

Experiment and replicate		Average green weight		
		Total	Seed only	Viscin only
			-- mg --	
1	1	2.0	1.2	0.8 (40%)
	2	2.2	1.6	0.6 (27%)
	3	1.7	1.1	0.6 (35%)
	4	1.9	1.4	0.5 (26%)
2	1	4.3	2.3	2.0 (47%)
	2	4.3	2.3	2.0 (47%)
	3	3.6	1.4	2.2 (61%)
	4	3.1	1.5	1.6 (52%)

#### MOISTURE CONTENT OF SEEDS AND VISCIN

In studies of A. campylopodum, fresh, mature seeds weighed 2.6-3.0 mg, while seeds dried over drierite weighed 1.6-2.0 mg.

Green seeds	Dried seeds		Basis
	--	Ave. wt. (mg) --	
2.6		2.0	350 seeds
2.7		1.6	70 "
2.7		1.8	250 "
3.0		--	80 "
2.9		--	30 "



In an experiment on fresh seeds of A. campylopodum, the moisture content was determined for 10 batches of 100 seeds each. Moisture content varied from 23% to 47%, with 8 of 10 batches having between 30-45% M.C. Moisture content was calculated by:

$$\frac{\text{green weight} - \text{dried weight}}{\text{green weight}} \times 100$$

The moisture holding capacity was tested for seeds and viscin separately and for intact seeds (each datum based on 10 seeds):

	Seeds without viscin		Viscin only	
	dry	soaked	dry	soaked
	-- mg --	--	-- mg --	--
Rep. 1	1.2	2.2	0.8	12.2
2	1.6	2.2	0.6	20.1
3	1.1	2.1	0.5	12.7
4	1.4	2.1	0.5	21.7
Average	1.3	2.2	0.6	16.7

Similar measurements on intact seeds show a weight change from 2.8 mg for fresh seeds, to 21.7 mg for seeds soaked 3 hours in water:

	Fresh seed wt.	Soaked seed wt.
	--mg--	--mg--
Replication 1	2.7	22.7
2	2.4	27.8
3	2.8	34.7
4	3.3	24.7
5	2.5	16.1
6	2.0	20.1
7	3.5	14.9
8	3.2	12.6
Average	2.8 mg	21.7 mg

Clearly the enormous water-holding ability of viscin is an important characteristic of species Arceuthobium, and undoubtedly accounts for their success in areas of low moisture or high evapo-transpiration potential.

We tested the ability of viscin to repeatedly absorb water. Seeds were tested in November, about 3 months after seeds had been harvested and stored at 1.5° C. Fifty seeds (5 replicates of 10 each) were placed on glass, and a similar set placed on filter paper. After 50 wet-dry cycles over 44 days, 26 seeds on filter paper and 16 seeds on glass still swelled normally. At no time was there evidence of irreversible hardening of the viscin. On those seeds which failed to swell after a number of cycles, the viscin appeared to slowly disintegrate, covering progressively less of the seed surface. Even these seeds, however, could be easily moved about the dish when wetted, and they adhered strongly when dried.

In another set of experiments, we recorded weights of A. campylopodum seeds before, during, and after 5 dry-wet cycles. The following tabulation shows the weight of seeds at each weighing (each datum is an average of 20 seeds).

	Experiment 1	Experiment 2
	-- mg --	--
Initial dry	3.3	3.4
First wet	36.0	32.0
Second dry	3.3	--
Second wet	30.0	20.7
Third dry	3.2	--
Third wet	17.2	17.4
Fourth dry	3.1	--
Fourth wet	--	11.0
Final dry	--	2.7

These data show a loss of viscin per se, and an increasing decline in the ability of viscin to absorb water. The final dry weight is about 85% of the original dry weight, whereas the final wet weight is only about 25% of the original wet weight.

A similar experiment was done comparing the behavior of viscin in 2 Molar solutions of NaCl and glucose.

Cycle	In H <sub>2</sub> O	In 2M NaCl	In 2M Glucose
	--	-- mg --	--
Initial	3.0	2.5	3.1
First wet	46.8	35.6	58.5
Second dry	2.7	5.5	7.6
Second wet	--	--	--
Third dry	--	--	--
Fourth wet	18.1	5.6	26.4
Fourth dry	2.5	2.8	2.6
Fifth wet	9.1	5.0	15.1
Fifth dry	2.4	2.7	2.5
Sixth wet	9.2	4.6	13.4
Sixth dry	2.5	2.8	2.5

Seeds in salt solution absorbed less water than did those in water, but the dry weight of salt-soaked seeds remained higher than the original dry weight. I have no explanation for this, other than it possibly indicates simple osmotic forces. Seeds in glucose solutions absorbed more water than the water controls throughout the experiment. Dry weight of glucose-soaked seeds and the water controls were not different.

Miscellaneous experiments of viscin showed the following:

1. Swollen seeds dropped into boiling water for 5 minutes remained swollen and "normal" looking.
2. Seeds wetted and placed under a lamp at 35° C dried and swelled 12 times with no apparent loss of function.
3. Mistletoe seeds oven dried (104° C) 24 hours had normal swelling response when subsequently placed in water.
4. Viscin on dead seeds absorbed water normally.
5. When fully expanded seeds of A. campylopodum were placed over drierite, the viscin lost 75% of its moisture in 6 hours.

# SEED DORMANCY, STORAGE, AND GERMINATION

As with seeds of many other genera, Arceuthobium seeds generally exhibit a post harvest dormancy (Beckman 1964). A. vaginatum is an exception, germinating soon after maturity (Hawksworth 1961). In our studies, seeds of A. campylopodum germinated more quickly with increasing time since seeds were harvested--a phenomenon called "germination readiness" by Beckman (1964). These data from a 1970 test show: (Each germination test utilized 300 seeds (3 replications x 100 seeds)).

Date of harvest	Date of germ. test	Days to max. germ.	Final germ. (%)
9/10	9/30	36-42	16-22
"	10/9	27-33	26-30
"	11/10	17-25	17-24
"	11/19	12-2	22-23
"	12/4	11-18	17-21
"	12/23	5-23	11-26

Another test in 1973 compared germination readiness for two species of Arceuthobium:

Days after harvest	Days to peak germination	
	<u>A. campylopodum</u>	<u>A. tsugense</u>
8	60	--
11	--	35
29	45	--
51	--	34
60	30	--
67	--	18
90	13	--

Germination of seeds of A. campylopodum was better in the light than in the dark under two temperature regimes. (Each datum based on three replications of 50 seeds).

	10° C		20° C	
Dwarf mistletoe	light	dark	light	dark
<u>A. campylopodum</u>	45%	19%	40%	32%
<u>A. tsugense</u>	30%	13%	38%	17%

These data confirm results of Beckman (1964) for A. campylopodum, and by Scharpf and Parmeter (1962) for A. occidentale.

Both Scharpf and Parmeter (1962) and Wicker (1962) state seed germination to be lower than corresponding viability tests using triphenyl tetrazolium chloride (TTC). This suggests that, under optimum conditions, germination could be improved.

## RADICLE GROWTH

Radicle growth rates of seeds of A. campylopodum and other species were measured in many experiments over a 10-year period. The following data show rates of radicle elongation per day for 5 regimes: constant low light (350 f.c.) and constant dark--both at 2 temperatures, and a 12-hour light-dark alteration. (Each datum based on 100 seeds).

Temperature	Light	Dark	12hrs, light-dark
10° C	53	24	--
20° C	92	77	45

In contrast, Bonga (1965) reported that light-temperature regimes had no effect on radicle growth of A. pusillum.

Radicle elongation of seeds of A. campylopodum was greater for the first 2 weeks than for the second 2 weeks:

Temperature	Radicle growth per day
10° C	39/day at 14 days
20° C,	29/day at 34 days
	75/day at 14 days
	59/day at 34 days

Beckman (1964) reported a similar trend of slower growth of older seeds of A. campylopodum.

Age of seeds	Growth/day
0-30 days	196
31-60 "	133
61-90 "	113
91-120 "	23

Radicle elongation of seeds of A. abietinum was determined at 4 temperatures, 32 days after the initiation of germination, and showed best growth elongation at 20° C.

Temperature	Growth/day
15° C	43
20° C	69
25° C	41
30° C	12

The reduction in growth rate past optimum temperature probably reflects the same physiologic stresses (such as high respiration, protein breakdown) that attend any plant that is too warm.

Data reported here are for seeds on glass slides placed in light-boxes within growth chambers and measured periodically with a calibrated dissecting scope. Bonga (1972) noted reduced germination and radicle growth at relative humidities of 90% or below.

An attempt was made to determine the rates of elongation in various combinations of relative humidity and temperature for several species of Arceuthobium. Various relative humidities were developed in flasks over solutions of polyethylene glycol. The growth rates, expressed as micrometers per day are shown for 4 species of Arceuthobium (Table 3).



Table 3. Rates of radicle elongation for 4 species of Arceuthobium at 12 temperature-relative humidity regimes.<sup>1</sup>

Relative humidity	<u>A. abietinum</u>			<u>A. campylopodum</u>			<u>A. douglasii</u>			<u>A. tsugense</u>		
	10°	15°	20°	10°	15°	20°	10°	15°	20°	10°	15°	20°
97%	60	68	72	66	64	64	60	--	68	50	62	56
83%	64	--	64	66	--	64	56	--	72	48	--	74
68%	60	--	--	72	--	62	--	--	62	52	--	--
18%	64	--	--	69	--	64	54	--	64	48	--	--

<sup>1</sup>The many data-less cells are the result of fungal contamination.

The data show a trend toward greater growth at higher temperatures and higher relative humidity. However, the great variability found in seed performance, and the large number of data-less cells prevent conclusive statements of radicle growth under these experimental conditions.

In our inoculation work, using small seedlings, we found that germinated seeds often fell off, so we tested various adhesives, finding polyvinyl acetate to be best. Comparative tests with A. campylopodum seeds with viscin only, and viscin plus polyvinyl acetate, showed that polyvinyl acetate did not influence radicle growth.

Seeds of three species of Arceuthobium were "glued" on the edge of upright glass slides and placed in darkened tubes with light from a point source at one end of the tubes. (Each datum based on 50 seeds).

Species	<u>Phototropism</u>		
	negative	positive	neither
<u>A. americanum</u>	90%	0%	10%
<u>A. campylopodum</u>	97%	0%	3%
<u>A. douglasii</u>	81%	0%	19%

Geotropistic responses were determined with similar methods. While the results were less clear-cut, there seemed no pattern of growth that could be interpreted as either negative or positive geotropism:

Species	<u>Radicle growth</u>		
	upward	downward	neither
<u>A. americanum</u>	16%	13%	71%
<u>A. campylopodum</u>	11%	9%	80%
<u>A. douglasii</u>	4%	24%	72%

Efforts to evaluate thigmotropistic responses were not completed, but most species of Arceuthobium seem to display a positive thigmotropistic response.

#### INFLUENCE OF LIGHT AND TEMPERATURE ON INFECTION AND SUBSEQUENT GROWTH OF DWARF MISTLETOE AND HOST TREES

Dwarf mistletoes were called a "light-loving plant" by Weir (1916) because of the rapid development of the epidemic when a stand is partially thinned. In contrast, Wagener (1961) reported that "partial sunlight is more favorable to the establishment of dwarf mistletoe on the host than relatively full or continuous sunshine." Wagener also reported that "robust" dwarf mistletoe development occurred at lower light levels:

<u>Estimated light intensity</u>	<u>% of infections that were "robust"</u>
Strong	2
Good	21
Medium	36
Fair	41

Wagener concluded that "if high light intensity is beneficial to the dwarf mistletoe, it must be exerted indirectly through the effect on the host tree or through the general influence on site conditions."

Our first greenhouse experiments were done with potted seedlings at three levels of shading. In the first experiment, each pot contained one uninfected seedling of ponderosa pine, and one seedling inoculated with germinated seeds of A. campylopodum. In the second experiment, pots had one infected pine seedling only. Tree heights, and the number and length of dwarf mistletoe aerial shoots were determined at intervals throughout the duration of the experiments. All pots were given a chill period (5° C, no light) from March to May of 1972, and then returned to the original chamber in the greenhouse. The results are summarized in Table 4.

Table 4. Mistletoe growth response for A. campylopodum on ponderosa pine at 3 levels of shading.

	Level of shading					
	Low shade		Med. shade		High shade	
	Experiment <u>1</u>	<u>2</u>	Experiment <u>1</u>	<u>2</u>	Experiment <u>1</u>	<u>2</u>
Height increase (mm)						
infected seedlings	5	17	10	14	7	10
uninfected seedlings	13	--	14	--	16	--
Increase in number of aerial shoots	50	17	21	4	33	11
Increase in length of aerial shoots (mm)	38	86	79	10	41	45
No. trees that became infected	4/4	4/6	4/4	2/6	4/4	5/6

The greatest number of aerial shoots were produced at the low shade level. The cumulative length of aerial shoots was greatest at low to medium shade, suggesting that mistletoe growth is best at medium or high light levels. Mortality of infected trees was: low shade, 20%; medium shade, 20%; and high shade, 60%. In contrast, no uninfected trees died.

Since this experiment dealt only with levels of light, another study was done in environment chambers, varying both temperature and light. One-hundred and eight ponderosa pine seedlings, each in a 3" plastic pot, were inoculated in April, 1971 with pregerminated seeds of A. campylopodum, and placed in one of three chambers (36 per chamber). Twenty-five were re-inoculated because the seeds died. All three chambers had a 14-hour day, 10-hour night. Night temperatures were 5 degrees below day temperatures. The light and temperature regimes were as follows:

	Light level	Temperature ( $^{\circ}$ C)	
	(f.c.)	Day	Night
Chamber #1	1500	25	20
Chamber #2	1500	20	15
Chamber #3	700	25	20

Results of this study are as follows:

#### Infection

The highest number of seedlings became infected at the low temperature-high light regime (22 of 36 trees). These data agree with Scharpf's report (1969) on the infection rate of Digger pine (Pinus sabiniana) and Monterey pine (P. radiata) by A. occidentale. He reported infection at

constant temperature regimes of 16 and 21 $^{\circ}$  C, but was best at 13 $^{\circ}$  C in an outdoor environment. The high temperature-high light chamber yielded the lowest number of infected plants (13 of 36).

#### Tree growth

Tree growth was best at high temperature regimes, with the best elongation growth at the low light level for both infected and uninfected trees.

Regime	Height increase	
	Uninfected	Infected
High temp, high light	8 mm	7 mm
High temp, low light	17 mm	13 mm
Low temp, low light	3 mm	3 mm

Also, it appears that the greater the tree growth, the greater the influence of mistletoe on that growth. For example, in the regime most favorable for tree growth, the mistletoe caused the greatest reduction in growth of the infected trees. At the low temperature regime, there was no difference between height growth of infected and uninfected seedlings. Tree height growth was markedly less at low temperatures than at the two high temperature regimes:

	Low temp. High light	High temp. High light	High temp. Low light
	mm	mm	mm
June 1971			
uninfected	17	20	25
infected	16	21	19
Sept. 1971			
uninfected	18	22	39
infected	18	26	30
Jan. 1972			
uninfected	19	29	42
infected	19	26	32



Table 5. Results of tree and dwarf mistletoe growth under 3 temperature-light regimes.

	25-20° C 1500 f.c.	25-20° C 700 f.c.	20-15° C 1500 f.c.
No. trees infected	13 of 36	15 of 36	22 of 36
Total no. aerial shoots after 8 mos.	71	96	81
Mean no. aerial shoots per infected tree	5.5	6.4	3.7
Mean length of longest aerial shoot (mm)	18.4	23.6	13.4
Tree height growth (mm)			
- infected	1.6	2.2	0.9
- uninfected	1.9	3.2	0.9

### Aerial Shoots

While low temperature favored infection, high temperature favored mistletoe biomass production. The highest number of aerial shoots were produced at the high temperature-low light regime. This same regime also had the highest average number of aerial shoots per seedling and the longest aerial shoots. (Table 5).

An interesting sidelight was that aerial shoots continued to grow in length after the host trees become dormant. This has also been reported for *A. vaginatum* in the Southwest (Hawksworth 1961). Also, the growth of the aerial shoots corresponded to the growth behavior of the trees: trees which had grown the fastest before the onset of dormancy had the most mistletoe growth after the onset of dormancy. Because dormant trees must be under caretaker status, metabolically speaking, mistletoe growth must reflect the general prosperity of the dormant host tissue, suggesting that the internal nutritional status of the host attained prior to onset of dormancy is important in subsequent mistletoe growth.

Thus, temperature, rather than light, seems to be the main environmental factor influencing mistletoe growth on young seedlings. Better seed survival and infection rate resulted at lower temperature. Subsequent growth of mistletoe was favored by higher temperature. The role of light seems indirect, through its influence on tree processes of photosynthesis, and starch and fat storage.

### CONCLUSIONS

Fruit and seed development in species of *Arceuthobium* seem to proceed sequentially: fruit

capsule enlargement, seed elongation, viscin development and, finally, maturation of embryo and endosperm tissue. As shown in Table 2, 60-87% of the final weight of the fruits of 4 species of *Arceuthobium* was attained in the final 22 days of a fruit develops over an approximately 12-month period (Hawksworth and Wiens 1972). Thus, the most energetically costly development occurs immediately prior to maturation. In survival terms, the mistletoe doesn't pay a high cost for fruits lost to environmental hazards, such as predators.

Post-harvest dormancy was present in the species studied in this report. This characteristic reduces survival hazards of a germinated seed exposed to hot fall weather, severe winter cold, and host tissue which is far from succulent. The ability of the seeds to germinate and grow at low temperatures (as early as February) allows infection to occur before the onset of hot summer weather and the appearance of insect predators.

The probability of infection success is increased by the following traits: radicle elongation of 50-100 micrometers per day over a wide range of relative humidities, light intensities, and temperatures; negative phototropism and neutral geotropism (thus, if a seed is on the underside of a limb, it will still be likely to grow upward into host tissue.)

While successful infection seems to be primarily a cool weather event, once organic union with the host is achieved, mistletoe biomass production was greater at higher temperatures. At these higher temperatures, aerial shoot production was greater at lower light levels.

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# The Pollination Biology of *Arceuthobium americanum* in Manitoba<sup>1</sup>

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Abstract.--There is evidence that both entomophily and anemophily play a significant role in the pollination of *Arceuthobium americanum*. The most commonly trapped insects, a *Bradysia* sp. (Diptera) and *Formica* spp. (Hymenoptera) are thought to be involved in chance pollination of the mistletoe. Among the Coleoptera, *Hyperaspis binotata* (Coccinellidae) and *Cyphon* sp. (Helodidae) were frequently found bearing mistletoe grains on both male and female brooms. Where female brooms were isolated from male brooms only 9% of the insects trapped bore pollen, while 79% of the flowers were pollinated. These data, together with the recovery of airborne pollen at least 400 m from a source, indicate that anemophily is also a significant pollinating mechanism.

## INTRODUCTION

The mechanism of pollination in the genus *Arceuthobium*, has been studied since the beginning of the century. A number of floral characteristics suggest entomophily as the mode of pollination, including sessile anthers, spined pollen grains, the clumping of pollen in clusters of 20-40 grains, low pollen production, non-plumose stigma, visual prominence of the male inflorescence and the presence of a glistening stigmatic exudate (Gill and Hawksworth 1961, Player 1979). Additional entomophilous characteristics have been noted by Dowding (1931a) who made reference to the presence of a central cushion in the male flowers of *A. americanum* Nutt. ex Engelm. which bears pairs of glandular cells suggesting nectaries. However, Cohen (1968) in his anatomical study of the staminate flower observed nothing to support this conclusion, although Hawksworth and Wiens (1972) regard the structure as a nectary. Gregor et al. (1974) describe the "pungent, citric-like odor" of both male and female flowers of *A. americanum* which might present olfactory cues to insect visitors. Anthocyanins are found in all species of *Arceuthobium* with dark shoots and in more than half of the light-coloured ones, perhaps functioning as visual attractants to dipteran pollinators (Hawksworth and Wiens 1972). The stigmatic exudate of *A. abietinum* Engelm. was analyzed by Brewer et al. (1974) by thin-layer and gas-liquid chromatography. Flowers produced large (0.24  $\mu$ l) droplets of highly concentrated nectar (52-92%) which Brewer

et al. (1974) concluded was probably a strong insect pollinator attractant.

Reports of hymenopterous insects associated with *A. americanum* were made by Weir (1915), and Dowding (1931b) reported both small flies and ants to be the pollinators of *A. americanum*. Kuijt (1955) also observed ants on the male inflorescences of *A. americanum*. Following these observations systematic studies of the insect fauna associated with *Arceuthobium* species were conducted. Stevens and Hawksworth (1970) in a review of the literature concerning the insects and mites associated with dwarf mistletoes concluded that the most common insect pollinator associated with *A. vaginatum* subsp. *cryptopodum* (Engelm.) Hawksw. & Wiens was a thrips of the genus *Frankliniella*, whereas on *A. americanum* small flies such as *Bradysia* sp. (Sciaridae) were common early in the season and succeeded by ants toward the end of the flowering period. The number of possible hymenopterous and dipteran pollinators of *A. americanum* was extended by Gregor et al. (1974) and Penfield et al. (1976) who collected more than 200 insect species bearing *Arceuthobium* pollen, although relatively few species were trapped from mistletoe of both sexes. Only 13 species were considered important and these differed to some extent for each *Arceuthobium* species. The pollinators of *A. americanum* included the ant, *Formica fusca* L. (Formicidae) and the dipterans *Philygria debilis* Lw., *Protophormia terraenovae* (R.-D.) (Calliphoridae), and several *Hylemya* species, *H. ceralis* (Gillette), *H. cinerella* (Fallen) and *H. platyura* (Meigen) (Anthomyiidae).

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Baker (1981) conducted a number of exclusion experiments, using screen bags of 0.8, 2.2 and 4.0 mm mesh and uncovered plants for control, to determine the mode of pollination of *A. pusillum*. Insects were trapped from female witches' brooms,

on screen traps and sticky board traps. Greater fruit set was recorded on plants covered with 4.0 mm mesh than on branches with smaller screens.

A number of studies to determine the concentrations of airborne mistletoe pollen have been conducted using vaseline-coated microscope slides placed at varying distances from male infections (Gregor et al. 1974, Penfield et al. 1976, Baker 1981). The small number of pollen grains trapped between 2.5 cm and 5 m from male flowers led Gregor et al. (1974) and Baker (1981) to conclude that A. americanum and A. pusillum were primarily insect pollinated. Penfield et al. (1976) trapped pollen of A. cyanocarpum Coulter & Nelson up to much greater distances from the nearest male flowers. While similar quantities of A. americanum and A. cyanocarpum pollen were trapped over their respective flowering periods, almost five times as much pollen from A. vaginatum was trapped. Penfield et al. (1976) concluded that both wind and insects effected pollination but suspected entomophily of being the primary pollinating mechanism.

Player (1979) studied the pollination of A. douglasii Engelm, and A. strictum Hawsw. & Wiens and found insect visitors were rare to the female flowers of both species. On the other hand laboratory studies indicated that pollen was easily liberated by wind. For continuous trapping of pollen, Player (1977) used square rods which had a higher calculated sampling efficiency at low wind speeds than the theoretical sampling efficiency of microscope slides. However, pollen dispersal was only measured within short distances from male flowers. There was relatively low seed set (2% or less) on female plants screened with mesh 0.5 mm and 1.0 mm in comparison with 11 to 62.5% seed set on uncovered plants. Player concluded that a large component of the wind with its pollen load would likely be forced around the cages. Plants bagged to prevent access of pollen either by insect or wind had no fruit set, ruling out the possibility of apomixis. Player (1979) argued that the pollination of Arceuthobium is principally an anemophilous system and based his conclusions on the lack of insect visitors and the relative abundance of airborne pollen.

In Manitoba dwarf mistletoe affects large areas of jack pine and spruce forest, especially in the south of the province in resort areas, where the infected trees are aesthetically displeasing and the dead and dying brooms are a potential fire hazard. A clarification of the pollination mechanism of Arceuthobium might be of significance in the development of appropriate control measures. In studies in the United States no specialized and few regular insect visitors have been found, while in Manitoba no systematic observations have been conducted on the insect fauna associated with dwarf mistletoes. Insects were therefore trapped and examined for evidence of pollen to confirm or reject their potential role as vectors.

The majority of studies of airborne pollen concentrations have employed microscope slides coated with petrolatum to form an adhesive surface. The levels of airborne Arceuthobium pollen measured with this method have been low. Moreover, most

dwarf mistletoe pollen trapped on slides has been in the immediate vicinity of male flowers while reports of long distance travel of pollen have been rare. In the present study concentrations of airborne dwarf mistletoe pollen and medium distance transfer were investigated using cheesecloth traps.

## METHODS AND MATERIALS

### A. Insects

Insects were trapped from dwarf mistletoe brooms using a beat net and kill jars for three consecutive seasons 1981 - 1983.

In 1981 three sites were chosen so that insects could be sampled from different environments:

1. Exposed lakeshore.
2. Sheltered, closed forest
3. Open forest

At each site, insects were trapped from female and male brooms at three times, morning (10.00 a.m. to 12.00 noon), noon (12.00 noon to 2.00 p.m.), and afternoon (2.00 p.m. to 4.00 p.m.), during the course of three days, in May, 1981. In 1981, 155 brooms were sampled; 86 male and 69 female.

A preliminary analysis indicated that the numbers of insects trapped by site, day and time of sampling were not significantly different, whereas a significant difference existed between the numbers of insects trapped from male and female brooms. Insects bearing pollen and trapped from female brooms were of most interest in the study, therefore in 1982 more female than male brooms were sampled, 73 female as opposed to 30 male. Insects were sampled from the three sites on 5 days in May, 1982.

Within the forest, where male and female brooms were in close proximity, insects with pollen adhering to them were trapped from brooms of both sexes. To identify pollen-bearing insects that might seek and pollinate female plants of A. americanum, five young jack pines with female dwarf mistletoe infections that were situated at least 10 m from the nearest male infection were sampled in the final season. Insects were trapped at weekly intervals from the start of the flowering period (April 26) until the male flowers dehisced, at the end of May.

### B. Wind

In 1982 and 1983 cheesecloth traps were used to collect airborne pollen. Cheesecloth was stretched over a square wire frame 15 x 15 cm and supported by 2 m bamboo poles. A 9 x 9 cm square from the centre of each trap was later scanned under a dissecting microscope for evidence of airborne mistletoe pollen. Sizes of pollen clusters and total numbers of grains per trap were recorded.



In 1982 two sites were chosen where stands of dwarf mistletoe-infected jack pine flanked areas which had been clear cut. Traps (3 replicates) were erected at 25 m, 50 m, and 100 m, from the pollen source. The cheesecloth was replaced six times at 24-hour intervals and twice at 4-day intervals. Before the male flowers opened in 1983, traps were erected 100 m, 200 m, 300 m, and 400 m from the pollen source at two similar sites. The cheesecloth traps were examined at the end of the seven week pollination period, June 7, 1983.

In 1983 five young jack pines bearing female dwarf mistletoe brooms established in a clear cut area were selected and marked. Two traps were erected by each tree on April 19, 1983 and changed at weekly intervals for the duration of the flowering period. Insects were trapped at weekly intervals from the same trees. At the start of the pollination period the number of developing fruits on 5 mistletoe plants on each tree plus one control tree from which insects were not trapped, were counted. In August, 1983, the same plants were examined to determine the number of flowers fertilized in the spring of 1983.

## RESULTS AND DISCUSSION

### A. Insects

A preliminary list of the insects trapped from A. americanum is given in Table 1. The four orders Diptera, Hymenoptera, Coleoptera and Hemiptera comprised almost 95% of the insects trapped. Thysanopterans were found relatively frequently (28, 5.7%) in 1981, four of which had a single grain adhering to them. Only one was found in 1982 and none in 1983. In 1981 three of the trapped insects belonged to the order Homoptera, in 1982 none and in 1983 two. Two microlepidopterans were trapped in both 1982 and 1983. These orders, Thysanoptera, Homoptera, and Microlepidoptera have been omitted from further analysis on account of their scarcity.

In 1981 and 1983 insects of the orders Diptera and Hymenoptera were trapped most frequently (Figs. 1, 3) while the orders Hymenoptera and Coleoptera were represented more frequently in 1982 (Fig. 2). In all three seasons the majority of the dipterans were Bradysia sp. (Sciaridae) (1981, 55%; 1982, 36%; and 1983, 54%) whereas Formicidae, Formica spp. (fusca and rufa groups) and Tapinoma sessile, comprised most of the Hymenoptera (1981, 70%; 1982, 61%; and 1983, 48%). The coccinellid, Hyperaspis binotata was especially abundant at Site 2 in 1981 and comprised 38% of all the coleopterans trapped in that year. Other insect taxa included in the list of insects associated with A. americanum were isolated less frequently.

In both years more than 50% of all the insects trapped from male and female brooms combined bore at least one pollen grain, although the majority of pollen bearing insects were trapped from male brooms (Table 2). Of the insects trapped on male brooms in 1981 and 1982,

Table 1. Preliminary list of insects collected from brooms of Pinus banksiana induced by Arceuthobium americanum in Belair Provincial Forest, Manitoba, 1981-1983.

			1981	1982	1983
DIPTERA	UNIDENTIFIED	6 specimens	-	+	-
	CECIDOMYIIDAE	2 specimens	-	-	+
	CHIRONOMIDAE	2 specimens	-	-	+
	CHLOROPIDAE	1 specimen	+	-	-
	EPHYRIDAE	3 specimens	-	-	+
		<u>Phlygria debilis</u> Lw.	+	+	+
		<u>Psilopsa olga</u> Cresson	+	+	-
	MUSCIDAE	<u>Musca domestica</u> L.	+	-	-
	MYCETOPHILIDAE	1 specimen	-	-	+
	SCIARIDAE	<u>Bradysia</u> sp.	+	+	+
		<u>Corynoptera</u> sp.	+	-	-
		<u>Lycoriella</u> sp.	+	-	-
	TEPHRI- TIDAE	<u>Tephritis</u> sp. <u>araneosa</u> (Coquil- let)?	+	-	-
HYMEN- OPTERA	UNIDENTIFIED	15 specimens	-	+	-
	CHALCIDIDAE	3 specimens	+	-	+
	CHALCIDIDAE	1 specimen	-	-	+
	ENCYRTIDAE	1 specimen	+	-	-
	PTERMALIDAE	5 specimens	+	+	+
	BRACONIDAE	3 specimens	+	-	+
	FORMICIDAE	<u>Camponotus herculeanus</u> (Linne)	+	-	-
		<u>Dolichoderus taschenbergi</u> (Mayr)	+	-	-
		<u>Formica</u> sp., <u>fusca</u> group	+	+	+
		<u>Lastus sitkaensis</u> Pergande	+	-	-
		<u>Tapinoma sessile</u> (Say)	-	-	+
	SCELIDNIDAE	1 specimen	+	-	-
	TENTHREDINIDAE	2 specimens	-	+	+
	XYELIDAE	1 specimen	+	-	-
COLEOPTERA	UNIDENTIFIED	5 specimens	-	+	+
	BUPRESTI- DAE	<u>Chrysobothris</u> sp.	+	-	-
	CHRYSO- MELIDAE	<u>Calligrapha</u> sp.	-	+	-
	COCCIN- ELLIDAE	<u>Coccinella</u> sp.	-	+	-
		<u>Coccinella transversoguttata</u> Fald.	-	+	+
		<u>Hippodamia tridecimpunctata</u> (L.)	+	+	-
		<u>Hyperaspis binotata</u> Say	+	-	-
		<u>Mulsantina hudsonica</u> Casey	+	-	-
		<u>Scymnus</u> ( <u>Pullus</u> ) <u>lacustris</u> Lec.	+	+	-
	CURCULIONIDAE	1 specimen	+	-	-
	ELATERIDAE	<u>Ctenicera triundulatus</u> (Rand.)	+	+	+
	HELDIDAE	<u>Cyphon</u> sp.	-	+	+
	LATHRIDII- DAE	<u>Corticaria</u> sp.	+	+	-
	MELYRIDAE	<u>Amecocerus</u> sp.	+	+	-
HEMIPTERA	LYGAEIDAE	<u>Kleidocerys resedae</u> (Parzer)	+	+	-
		<u>Ischnorrhyncus</u> sp.	-	+	-
		<u>Nysius ericae</u> (Schilling)	-	+	-
	MIRIDAE	<u>Lygus</u> sp.	-	-	+
HOMOPTERA	CICADELLI- DAE	1 specimen	-	-	+
	PSYLLIDAE	<u>Trioxa obtusa</u> Patch <u>T. tripunctata</u> (Fitch)	+	-	-
MICROLEPIDOPTERA UNIDENTIFIED			-	+	+
THYSANOPTERA UNIDENTIFIED			+	+	-

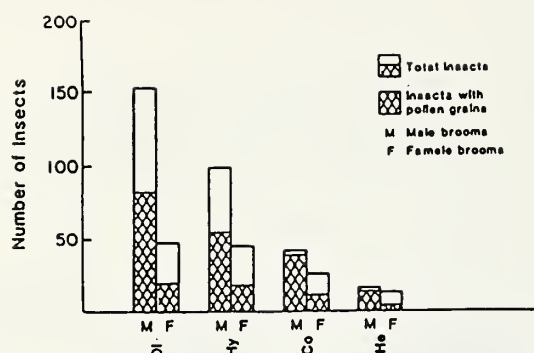


Fig. 1. Diptera, Hymenoptera, Coleoptera, and Hemiptera trapped from brooms of *Pinus banksiana* induced by 86 male and 69 female infections of *Arceuthobium americanum* in Belair Provincial Forest, Manitoba, 1981.

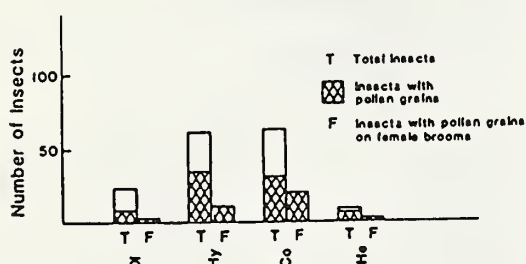


Fig. 2. Diptera, Hymenoptera, Coleoptera, and Hemiptera trapped from brooms of *Pinus banksiana* induced by 30 male and 73 female infections of *Arceuthobium americanum* in Belair Provincial Forest, Manitoba, 1981.

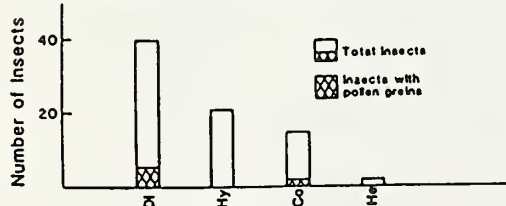


Fig. 3. Diptera, Hymenoptera, Coleoptera, and Hemiptera trapped from five brooms of *Pinus banksiana* induced by female *Arceuthobium americanum* in Belair Provincial Forest, Manitoba, at six weekly intervals in 1983.

Table 2. Percentage of insects bearing mistle-toe pollen from brooms of *Pinus banksiana* induced by *Arceuthobium americanum* in Belair Provincial Forest, Manitoba.

YEAR	NUMBER OF BROOMS		MEAN NUMBER OF INSECTS PER BROOM		% INSECTS WITH POLLEN	
	M	F	M	F	M	F
1981	86	69	3.7	2.4	60	34
1982	30	73	2.0	1.4	72	39
1983		30		2.7		9

60% and 72% respectively carried pollen, whereas for the same seasons, 34% and 39% respectively of insects trapped on female brooms bore pollen (Table 2). In 1983 the number of pollen-bearing insects trapped on female brooms was considerably lower, 9% (Table 2), than in either 1981 or 1982. Fewer brooms were sampled in 1983 but the mean number of insects per broom was 2.6, which was comparable to the numbers collected in 1981.

Several of the insect families collected in Manitoba were represented in earlier studies in the United States. Of the Diptera, Mycetophilidae (Fungus gnats; one specimen trapped), was the only family not previously associated with *A. americanum* (Gregor et al. 1974, Penfield et al. 1976). The majority of the Diptera trapped belonged to families characterized by sucking mouthparts (Cecidomyiidae, Chironomidae, Ephydriidae, Mycetophilidae and Sciaridae). The small fly *Bradysia* sp. (Sciaridae) was collected most frequently from both male and female brooms in Manitoba and was commonly well dusted with pollen. Sciaridae has been described as the chief anthophilous dipteran family (Proctor and Yeo 1972).

The families Braconidae, Encyrtidae, Pteromalidae, Scelionidae, and Tenthredinidae (Hymenoptera) were poorly represented in the Manitoba study, but have been found in association with *A. americanum* in Utah or Colorado (Gregor et al. 1974; Penfield et al. 1976). The super-family, Chalcidoidea and family Chalcididae plus the family Xyelidae are reported in this association for the first time. Chalcid wasps have been recorded at flowers (Proctor and Yeo 1972), and larvae of the Xyelidae are known to feed on the staminate cones of pines (Rose and Lindquist 1973). However, the family in the Hymenoptera best represented was the Formicidae. Ants regularly collect nectar from flowers but are unlikely to effect cross-pollination as the workers responsible for nectar collection, are wingless and have to reach flowers by crawling up the trunks of trees and stems of flowers (Proctor and Yeo 1972). Several genera (*Lasius*, *Camponotus*, *Formica*) and two species (*Formica* sp. *fusca* group, *Tapinoma sessile*) have been trapped in the United States as well as in Canada. Many ants were found covered with *A. americanum* pollen or with it clustered about the mouthparts, but pollen was not observed on ants trapped from female dwarf mistletoe that was isolated from male infections in 1983 (Fig. 3).

Among the Coleoptera, Coccinellidae, Elateridae and Melyridae were collected in both the United States and Canada; the Melyridae is the one exclusively anthophilous family among this group (Proctor and Yeo 1972). Many specimens of *Ctenicera triundulatus* (Elateridae) were found with abundant pollen adhering to them, but the family is known to feed destructively on flowers, offsetting the value of any chance pollination effected by the damage they cause (Proctor and Yeo 1972). Different species of the genera *Ctenicera* (Elateridae), *Mulsantina* and *Scymnus* (Coccinellidae) have been reported from both Colorado and Utah, United States, and from Manitoba, Canada. *Coccinella transversoguttata* represents the one coleopteran species that is presently known as a denizen of *A. americanum*.



infected pine both in the United States (Utah) and Manitoba. As a family the Coccinellidae are primarily an entomophagous group but nectar and pollen also commonly provide an alternate food source when insect food is scarce, permitting survival and immediate resumption of oviposition when insect prey reappear (Hagen 1962, Hodek 1973). Ladybird beetles (Coccinellidae) were not considered important pollinators of dwarf mistletoe by Gregor et al. (1974) or Penfield et al. (1976); however, Hyperaspis binotata was one of the most common insect visitors to A. americanum in Manitoba, and pollen grains were frequently found about the mouthparts and adhering to the ventral surface. The marsh beetle, Cyphon sp. (Helodidae) was also frequently found on brooms and well dusted with pollen in the final two years of the study.

Kleidocerys resedae was the only hemipteran recorded with any frequency from A. americanum in Manitoba; the family (Lygaeidae) and genus have not been recorded in this association elsewhere. Members of this order have often been recorded at other flowers (Proctor and Yeo 1972). The piercing sucking mouthparts suck up the juices of the plants but the bugs may become dusted with pollen and effect chance pollination as they move from flower to flower (Proctor and Yeo 1972). K. resedae, well dusted with pollen, were trapped from both male and female brooms from Site 1 in 1981 (Fig. 1).

A number of insects reported as anthophilous and implicated in the pollination of flowers were found in the immediate vicinity of A. americanum in Belair Provincial Forest. While the majority of insects, including those that were well dusted with pollen, were trapped on male brooms, a significant number were taken from female brooms that were in close proximity to male brooms (Figs. 1, 2). However, insects with pollen were rarely trapped from female brooms that were isolated from male brooms (Fig. 3).

## B. Wind

Total pollen counts and number of clusters of pollen (dispersal units) trapped at 25 m, 50 m, and 100 m from the source (1982) varied greatly, but with no consistent pattern, between sites, ranging from 0 to 60 for total pollen count per 24 hour period and 0 to 18 for number of dispersal units over a similar interval of time (Table 3). Considerable variation between sites and traps was also observed in 1983 (Table 4). However, analysis of variance to determine the effect of site and distance revealed that the number of pollen grains and dispersal units trapped at each distance did not vary significantly between the two sites in 1982 so the data were combined. Although the numbers of dispersal units (each of which can only effect the pollination of a single female flower) were found to increase slightly with distance from the source, the concentration of airborne pollen was relatively uniform over the distance measured. However, the percentage of single pollen grains was greater at 50 m and 100 m (Fig. 4). At 25 m, 64% of the dispersal units were single grains whereas at 50 m and 100 m 79% and 71% respectively were single grains. An ANOVA to determine the effect

of site and distance on the number of pollen grains trapped at 100 m to 400 m in 1983 revealed that significantly more pollen was trapped from site 1, exposed to sources both to the north and east, than at site 2 where pollen was dispersed from the east alone. Significantly more pollen clusters were trapped at 200 m and 300 m at both sites, although the highest percentage, 73%, of single pollen grains occurred in traps farthest from the source (Fig. 5). Smaller clusters are expected as distance increases but their numbers may also have risen as a result of the disintegration of dispersal units. From 100 m to 300 m the percentage of single grains was lower, ranging from 24-35% (Fig. 5).

Airborne pollen of A. americanum was found at greater distances from the source than might have been expected from previous studies (Gregor et al. 1974, Baker 1981). Player (1979) did not measure pollen dispersal beyond 7 m but Gregor et al. (1974) found no A. americanum pollen more than 1 m from source. Baker (1981) concluded that the numbers of airborne pollen grains of A. pusillum ( $0.4/\text{cm}^2/\text{year}$ ) trapped within 5 m of male flowers were too low to support the hypothesis of an anemophilous pollination mechanism. Penfield et al. (1976), however, trapped pollen of A. cyanocarpum up to 151 m from the nearest male flowers.

Higher numbers of dispersal units were trapped in 1982 in a two week period, in which sampling was conducted at several 24-hour intervals and two 4-day intervals, than in 1983 when the traps were exposed for the entire pollination period (Figs. 4, 5). A possible explanation of the disparity in the pollen counts between 1982 and 1983 is the washing of pollen from the traps by rain during the course of the seven week exposure.

Dips and peaks were observed in the concentration of airborne pollen in 1982 and 1983 suggesting that release is not uniform or continuous over the pollination period. The climatic data available failed to implicate either temperature or precipitation as the sole cause for arrest or retardation of pollen discharge. However, the sites were close to Lake Winnipeg, a large body of water which would no doubt influence local temperature and precipitation, while the study had to rely on the climatic records of Environment Canada Meteorological Office for Pine Falls, the closest meteorological station to the study sites and some 16 km distant. This may have obscured the relationship between airborne pollen concentrations and temperature and/or precipitation.

## POLLINATION SUCCESS

The experiment to determine the number of successfully pollinated flowers on trees isolated from male dwarf mistletoe infections was disturbed in a number of ways. Several tags were chewed, presumably by rodents, and several stems of mistletoe either eaten or dislodged. Some tags were chewed through and had fallen off making it impossible to make the second count on several plants. One entire tree and several tagged plants had died from other causes. Only 14 of the original twenty-five marked plants on the experimental trees and one of the five

Table 3. Total counts of pollen grains and dispersal units of Arceuthobium americanum recorded for Sites 1 and 2 at increasing distances from the source in Belair Provincial Forest, Manitoba, 12-25 May, 1982.

Date May 1983	Total Pollen Count*							Dispersal Units*					
	25 m		50 m		100 m		Total	25 m		50 m		100 m	
	Site		Site		Site			Site		Site		Site	
	1	2	1	2	1	2		1	2	1	2	1	2
12	0	34	26	4	9	1	74	0	3	2	4	4	1
13	10	0	2	12	0	60	84	1	0	2	3	0	1
14	3	11	4	7	4	2	31	3	10	4	7	4	2
15-18	9	56	33	50	50	51	249	8	34	16	45	35	43
19	3	2	0	1	0	2	8	3	2	0	1	0	2
20	20	14	13	36	11	19	113	4	9	5	15	6	16
21	10	11	4	8	53	21	107	5	6	3	8	18	12
22-25	184	61	89	63	249	88	734	65	30	52	34	56	25
Totals	239	189	171	181	376	244		89	94	84	118	123	102
Mean number per trap	80	63	57	60	125	81		30	31	28	39	41	34

\* Sum of 3 replicates.

Table 4. Total counts of pollen grains and dispersal units of Arceuthobium americanum recorded for Sites 1 and 2 at increasing distances from the source in Belair Provincial Forest, Manitoba, 19 April to 7 June, 1983.

	100 m		200 m		300 m		400 m	
	Site		Site		Site		Site	
	1	2	1	2	1	2	1	2
Total pollen count *	13	17	48	41	59	37	10	6
Dispersal units *	5	11	4	15	15	18	9	5

\* Count for one trap

on the control tree (from which no insect collection was attempted) remained at the time of the second count (Table 5). On the 15 surviving plants just 50% of the developing fruits counted in April reached maturity. Considerably more flowers appeared to have been pollinated in the spring of 1983 (272) than 1982 (99) (Table 5), although a count made in the spring of 1984 revealed that only 12%, 20 out of 172 developing fruits that were traced, had overwintered successfully. The reason for such low survival requires further study.

#### GENERAL DISCUSSION

There is evidence that both entomophily and anemophily play a role in the pollination of A. americanum.

Members of several anthophilous insect families, some well dusted with pollen, were found associated with dwarf mistletoe flowers. Ants of the genus Formica and Bradysia sp. (Scleridae) have commonly been reported as insect pollinators of A. americanum in earlier studies (Gregor et al. 1974, Penfield et al. 1976). In Manitoba, two coleopterans, Hyperaspis binotata (Coccinellidae) and Cyphon sp. (Helodidae), were frequently trapped and may effect a degree of pollination. However, the scarcity of H. binotata after 1981 and the absence of Cyphon sp. in the first year of sampling is perhaps indicative of a local and erratic population buildup in some insect species. Presumably, flowers such as A. americanum with no special adaptations to specific insect pollinators are placed at advantage if such a population explosion occurs in a pollinating insect species. None of the insects reported showed specific adaptations for the pollination of A. americanum; rather it would appear that a number of unspecialized insect visitors are responsible for chance pollen transfer in conjunction with nectar and/or pollen predation. However, in 1983 insects bearing pollen and trapped from female A. americanum isolated from male brooms were few, 9% of the total, although an appreciable percentage, 79%, of flowers with receptive stigmata were fertilized. The experiment had been disturbed, but the pollination success was high, suggesting that the wind as a pollination mechanism of A. americanum was effective.

Pollen was trapped up to 400 m from the source over a clear cut area which provides evidence that, in spite of its entomophilous characteristics, presence of spines and tendency to cluster, it is easily transported by the wind. At approximately 20  $\mu$  diam., A. americanum pollen falls within the



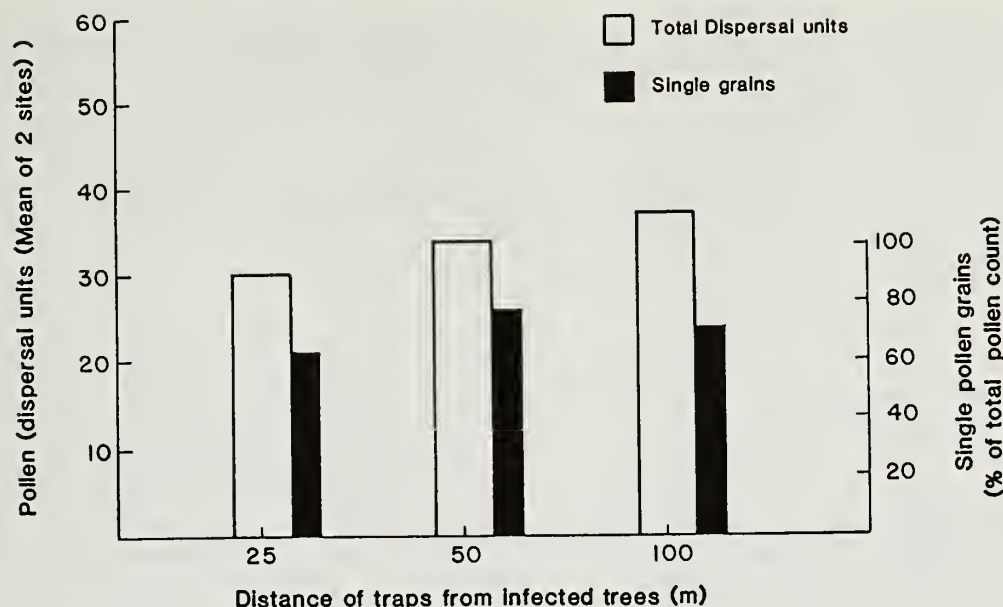


Fig. 4. Dispersal of *Arceuthobium americanum* pollen in Belair Provincial Forest, Manitoba, 1982.

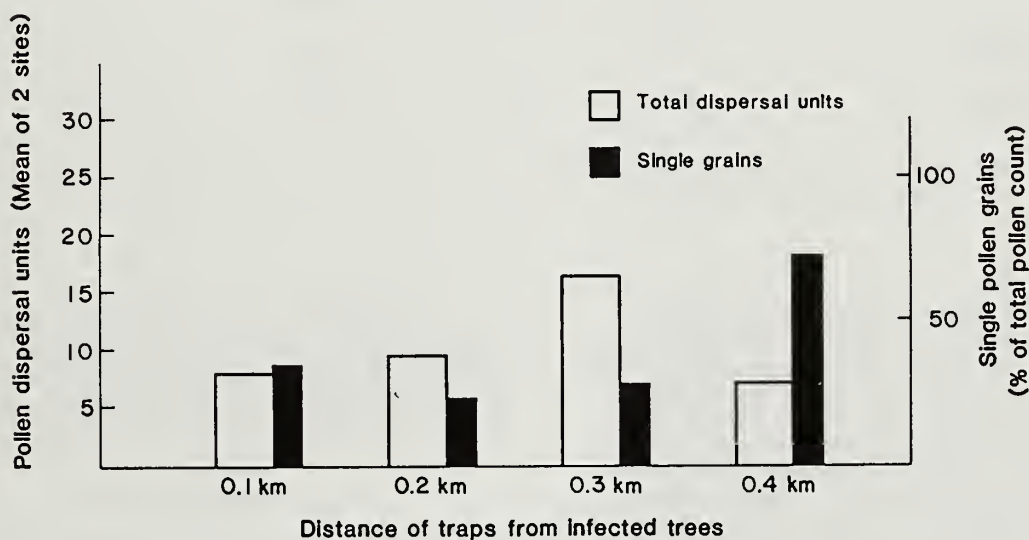


Fig. 5. Dispersal of *Arceuthobium americanum* pollen in Belair Provincial Forest, Manitoba, 1983.

size range encountered in most wind-pollinated species (Whitehead 1969).

#### EFFICIENCY OF TRAPPING AIRBORNE POLLEN

Most studies have concluded that both insects and wind are effective pollinators of *A. americanum*; opinions differ as to which is the primary mechanism (Baker 1981, Gregor et al. 1974, Penfield et al. 1976, Player 1979). Microscope slides, coated with vaseline or petrolatum, have been commonly used to

trap airborne spores and pollen and were employed by the supporters of entomophily (Baker 1981, Gregor et al. 1974, Penfield et al. 1976). However, microscope slides have poor theoretical collection efficiency at the low wind speeds observed within a forest, 0.0% at 1 m/sec and 46% at 10 m/sec (Player 1979). The problem is exacerbated when the spores in question are small (Gregory 1973), and it is most probable that the method has led to an underestimation of the airborne pollen load.

Table 5. Pollination success of tagged plants of female Arceuthobium americanum infected Pinus banksiana isolated from male infections in Belair Provincial Forest, 1983.

Tree	Plant	First count (April 1983)	Second count (August 1983)		Third count (June 1984)
		Developing fruits	Mature fruits	Developing fruits	Mature fruits
1	1	2	0	32	*
	2	0	0	15	—
	3	0	0	43	0
	4	0	0	24	2
	5	0	0	6	0
3	1	3	0	13	8
	2	18	2	1	0
	3	7	2	1	0
	4	0	0	2	0
	5	4	1	2	0
4	1	1	1	0	0
5	2	45	35	31	—
	3	0	0	68	0
	5	19	11	22	—
CONTROL	2	0	0	12	10
TOTALS	15	99	50	272	20

\* Tags lost over winter.

The cheesecloth traps used in the present study avoided some of the problems associated with sticky slides. Air passes through the cloth trapping a proportion of the spined pollen grains on the cheesecloth fibers. The pollen grains of A. americanum are small (20  $\mu$ ) and probably carried in the airstream around a microscope slide unless the wind speed is high enough to give momentum sufficient for impaction of the spores. While the cheesecloth traps can only give an indication of the relative concentrations of pollen in the air it is felt that they may give a better estimation than the microscope slides.

In conclusion, there is sufficient evidence to suggest that both entomophily and anemophily are important pollination mechanisms of A. americanum depending on the local environment in which pollen transfer is taking place. Anemophily is most likely to be of significance in open forest and for medium distance transfer of pollen while within closed forest chance visits by unspecialized insect visitors will effect a degree of pollination; that is, "some plants make the best of both worlds" (Gregory 1973).

An experiment was conducted in the spring of 1984 to compare the relative efficiency of the cheesecloth traps used in this study with greased microscope slides. The data have yet to be analysed and no conclusions can be drawn at this time.

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# Insect-Dwarf Mistletoe Associations: an Update<sup>1</sup>

Robert E. Stevens and Frank G. Hawksworth<sup>2</sup>

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Abstract.--The last 10 years have seen a marked increase in the attention given insect-dwarf mistletoe relationships. Areas of interest include insects as pollinators, insects phytophagous on dwarf mistletoe shoots, flowers, and seeds, and combined insect-dwarf mistletoe effects in tree mortality. The latter category has been the subject of considerable work in the area of increased susceptibility of dwarf mistletoe-infected trees to bark beetles, especially Dendroctonus. Many insects of dwarf mistletoes have been identified but much more research will be needed before their potential as practical biological control agents can be assessed.

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In 1970, we reviewed arthropod-dwarf mistletoe relationships (Stevens and Hawksworth 1970), and identified 3 general kinds of associations between insects and dwarf mistletoes: (1) pollination of dwarf mistletoe by insects, (2) insects feeding on dwarf mistletoe, and (3) increased tree susceptibility to insect attack induced by dwarf mistletoe. The pertinent literature up to the mid-1970's was reviewed by Scharpf (1975).

The 1970s saw a marked increase in attention given to these associations, and it seems useful at this time to update our treatment of them. The 3 categories of relationships are still pertinent.

## INSECTS AS POLLINATORS OF DWARF MISTLETOES

In our earlier paper (Stevens and Hawksworth 1970), we noted that a variety of thrips, bees, ants, and flies had been reported to participate in dwarf mistletoe pollination. We speculated about the possibility of limiting pollination and dwarf mistletoe spread by controlling or repelling arthropod pollinators.

Since our 1970 paper, several studies (Gregor et al. 1974, Penfield et al. 1976, and Player 1979), have significantly broadened our knowledge about dwarf mistletoe pollination ecology, and several others have added important details (see Gilbert and Punter, these proceedings).

In the earliest of the major studies, Gregor et al. (1974), observed at least 25 species of insects on flowers of Arceuthobium americanum on Pinus contorta in Utah. Flies (Anthomyiidae, Syrphidae, Calliphoridae, Muscidae, and Ephydriidae), and ants (Formicidae), were the most common insect visitors. The authors felt that the evidence pointed toward pollination by insects (entomophily) being more important than that by wind (anemophily).

In the Colorado study by Penfield et al. (1976), over 200 species of insects were found bearing pollen of A. americanum, A. vaginatum, and A. cyanocarpum. However, the authors also acknowledged that wind was also likely an important element in pollination, with some pollen being dispersed up to 150 meters from its source. In this paper it was felt that ants and flies were the most important insect pollinators. The ubiquitous thrips (considered important by some workers) while numerous, were found to remain excessively long on male dwarf mistletoe flowers, arriving on female inflorescences too late to be effective in pollination.

Player (1979), conducting studies in the Douglas-fir forests of Utah (A. douglasii) and pine forests of northern Mexico (A. strictum), did not find an extensive entomofauna involved in pollination, particularly in the case of A. douglasii. He opted for anemophily as the general pollination mechanism in Arceuthobium.

In an unpublished report, Williams (1970) found unidentified thrips common on flowers of A. campylopodum infecting P. jeffreyi in San Diego County, California. Williams discusses more thrips activity in male than female flowers, which may be significant in light of the findings by Penfield et al. (1976).

Baker et al. (1978) briefly report studying the role of wind and insects in pollination of A. pusillum in Minnesota. A large fly and a

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beetle, both unidentified, "were found to carry large amounts of pollen." The authors considered wind pollination to be of minor importance.

In summary, there does not appear to be a clear case for either anemophily or entomophily. It seems likely both are involved. Given all this, limiting pollination via insect control and thus limiting dwarf mistletoe spread seems an unlikely prospect.

#### INSECTS FEEDING ON DWARF MISTLETOES

In Stevens and Hawksworth (1970), we listed a variety of kinds of insects that fed on dwarf mistletoes. Advances in this area since 1970 in the United States have mostly been limited to additional reports of occurrences of known species, and descriptions of species associated with dwarf mistletoe. However, the period also saw several U.S. Department of Agriculture-supported studies in Pakistan aimed at basic understanding of dwarf mistletoe-insect relationships on the Indian subcontinent.

Larvae of *Mitoura* (*Callophrys*) *spinetorum* are well-known as herbivores on dwarf mistletoe, and the adults are prized by butterfly collectors. According to Ferris and Brown (1981), the species "occupies a wide distribution throughout the western United States." Other recent reports of its occurrence are found in Austin (1981), Emmel and Emmel (1973), Holland (1974), Kohler (1980), Tilden and Hunter (1977), and Tunno and Dooling (1976).

Tietz (1972) repeats an earlier report of *M. johnsoni* (McCorkle 1962); however, he errs in calling the host *A. douglasii*. The main host of *M. johnsoni* is *A. tsugense*. We (Stevens and Hawksworth 1970) overlooked McCorkle (1962). Evidently *M. johnsoni* has a 1-year life cycle, and the larvae feed readily on *A. tsugense* and sometimes on other species of *Arceuthobium*. McCorkle (in Anonymous 1982) noted that, in an Oregon study, 28 larvae of *M. johnsoni* completely destroyed 106 of 144 *A. tsugense* shoots.

Another lycaenid butterfly, *Lycaenopsis cinerea* Edwards, was reported for the first time feeding on *A. americanum* in British Columbia (Ross et al. 1973).

Spittlebugs, often seen on dwarf mistletoe in the southwestern United States, were the subject of several reports in the 1970's and early 1980's (Fuller and Hostetler 1980, Lessard 1978, Lessard and Walters 1978, and Ragenovich 1980). In our earlier paper (Stevens and Hawksworth 1970), we commented on the nomenclature of the species reported to be on dwarf mistletoe, *Clastoptera obtusa* (Say). Specimens from a recent (1978) collection in Arizona were authoritatively identified as *C. distincta* Doering; we now presume that the citations of *C. obtusa* as a dwarf mistletoe associate were errors.

The work in Pakistan, conducted during the period 1973-1976 (Baloch and Ghani 1980), was an initial search for insects feeding on *A. minutissimum* and *A. oxycedri*, with an eye toward biological control. *Cosmotola inops* Prout, a geometrid, was the sole species found feeding on *A. minutissimum*. This insect has a 1-year life cycle, overwintering as 3rd and 4th instars. Two species of moths, *Dioryctria taiella* Amsel (Pyralidae), and *Prosinitis florivora* Meyrick (Blastobasidae), fed on *A. oxycedri*. Both species overwinter as larvae, and are univoltine. Other insects recorded from *A. oxycedri* (Baloch et al. 1977), include *Polydrusus obligatus* Fst. and *Systates* sp. (Coleoptera, Curculionidae); *Dolycoris indicus* Stal. (Hemiptera, Pentatomidae); and *Dichrocytus valesianus* (Hemiptera, Miridae). Nothing more is reported of this latter set.

In Colorado, Stevens et al. (1977) found larvae of a normally pine-feeding tortricid moth, *Choristoneura lambertiana ponderosana* Obraztsov, colonizing *A. vaginatum*. Late-stage larvae fed heavily on the dwarf mistletoe, and apparently normal, though smaller, moths were produced. Emergence of the moths that fed on dwarf mistletoe was delayed about 2 weeks.

Several new insect associates have been described from dwarf mistletoe since 1970. All but a thrips, *Frankliniella hawksworthii* O'Neill, which may be a pollinator, are presumed to feed on dwarf mistletoe shoots or flowers. These newly-described species and some described species first reported on *Arceuthobium* are listed in Table 1.

In a somewhat unusual context, Hawksworth and Wicker (1973) for the first time reported the common *Aspidiotus nerii* (Bouché) (Homoptera, Coccidae), as a dwarf mistletoe associate. This species and *Hemiberlesia rapax* (Comstock), both colonizing *A. hondurensis*, were infected with *Nectria flammula* (Tulasne) Dingley, a common parasite of scale insects, in Honduras.

Another insect-dwarf mistletoe relationship that has received little attention, but is may be significant in the mistletoe's life history is insect predation of seeds before and after germination. In inoculation studies with *A. vaginatum* in ponderosa pine in Arizona, nearly 70% of the planted seeds were removed or destroyed, mostly by undetermined insects (Hawksworth 1961). Insects are also involved in seed predation of *A. tsugense* in Oregon (Carpenter et al. 1979) and of several species of dwarf mistletoe in the Inland Empire (Wicker 1967). Studies to determine the insects involved in dwarf mistletoe seed predation are needed.

#### INCREASED TREE SUSCEPTIBILITY TO INSECTS INDUCED BY DWARF MISTLETOE

A major recent development in the understanding of the ecology of forest insects-- particularly bark beetles (Scolytidae)-- and

diseases has been the increasing awareness that these kinds of pest organisms often act together in causing tree mortality. Typically, pest complexes--involving both bark beetles and diseases--are the causes of tree death. The California Pest Damage Inventory has done much to clarify the complex interactions of insects, diseases, and environment in tree mortality (Byler 1978, Smith and Roettgering 1982).

The increase in the number of publications on this kind of association between insects and dwarf mistletoe shows the interest in the topic. When we completed our review 14 years ago (Stevens and Hawksworth 1970), we cited only 10 references. Today there are more than 100. Many of these publications report observational material only, but a few report quantitative data. Some of the most significant reports on association of dwarf mistletoes and insects in relation to tree mortality are summarized in Table 2.

The nature of the association between "primary" bark beetles (*Dendroctonus*) and dwarf mistletoes is still poorly understood. Many variables, including the host, locality, and environmental factors, influence the association. In some cases, the prevailing evidence is that dwarf mistletoe increases susceptibility to the insects (e.g., *Dendroctonus ponderosae* and *D. brevicornis* in ponderosa pine) but, in other instances, there seems to be little or no effect (e.g., *D. pseudotsugae* in *Pseudotsuga menziesii*), and in others, dwarf mistletoe-infested trees may be less susceptible (e.g., *D. ponderosae* in *Pinus contorta*). There are many reports and observations that "secondary" insect

species (*Ips*, *Melanophila*, etc.) attack mistletoe-weakened trees and hasten their death.

Twig beetles frequently infest and kill mistletoe-infected branches. Some specific records provided by Malcolm Furniss (personal communication 1982) are as follows: (1) *Pityophthorus schwartzii* Blackman on ponderosa pine infected by *Arceuthobium vaginatum* on the Coronado National Forest Arizona, and (2) *Pityophthorus deletus* Le Conte on limber pine infected by *Arceuthobium cyanocarpum* on the Dixie National Forest, Utah.

## DISCUSSION

Where can we look for added understanding of insect-dwarf mistletoe relationships, and where can these help in forest management? It would appear that pollination ecology is complex enough so that simple solutions to mistletoe spread will not come easily from this approach.

We have shown that several phytophagous insect species have been identified as likely biological control agents. Additional work on this management approach surely appears justified.

Finally, as forest management becomes more intensive in western North America, further attention to bark beetle-dwarf mistletoe relationships may provide clues to potential problem areas. It is encouraging to note that more and more foresters are viewing these pest problems as often complex, and not always resulting from actions of a single kind of organism.

Table 1. Species of insects newly reported to feed on dwarf mistletoes, 1970-1984

Insect	Host	Tree	Locality	Reference
<b>THYSANOPTERA</b>				
<b>Thripidae</b>				
<i>Frankliniella hawksworthii</i> O'Neil	<i>A. vaginatum</i>	<i>Pinus ponderosa</i>	Colorado	O'Neill 1970
<b>HEMIPTERA</b>				
<b>Miridae</b>				
<i>Neoborella canadensis</i> Kelton & Herring	<i>A. americanum</i>	<i>Pinus banksiana</i>	Alberta, Canada	Kelton & Herring 1978
<i>N. pseudotsugae</i> Kelton & Herring	<i>A. douglasi</i>	<i>Pseudotsuga menziesii</i>	Arizona, and New Mexico	Kelton & Herring 1978
<i>N. xanthenes</i> Herring	<i>A. americanum</i>	<i>Pinus contorta</i>	Colorado	Herring 1972
<i>Platylygus mexicanus</i> Kelton	"mistletoe" <sup>1</sup>	<i>Pinus leiophylla</i>	Durango, Mexico	Kelton & Knight 1970
<b>COLEOPTERA</b>				
<b>Scolytidae</b>				
<i>Pityophthorus arceuthobii</i> Wood	<i>A. globosum</i>	<i>Pinus montezuma</i>	Durango, Mexico	Wood 1971
<b>Mordellidae</b>				
Unidentified mordellid	<i>A. abietinum</i>	<i>Abies magnifica</i>	California	R. F. Scharpf, Personal Communication, 1983
<b>ORTHOPTERA</b>				
<b>Locustodea</b>				
<i>Malanoplus devastator</i> Scudder	<i>A. campylopodum</i>	<i>Pinus ponderosa</i>	California	R. F. Scharpf, Personal Communication, 1984

<sup>1</sup> Probably is *A. gillii* subsp. *nigrum* (F.G.H.).



Table 2. Reports and observations of dwarf mistletoe-insect associations in relation to tree mortality, 1970-1984.

Host and location	Insect and dwarf mistletoe association	Reference
<u>Abies</u> spp.		
California	Eighty percent of the mortality in red and white fir was due to a 3-organism complex, <u>Scolytus ventralis</u> , <u>Fomes annosus</u> , and dwarf mistletoe.	California Forest Pest Control Action Council, 1975
California	Dwarf mistletoe is a secondary cause of death in red and white fir; it weakens trees to the point that insects are attracted in sufficient numbers to kill them.	Gordon 1973
<u>Pinus banksiana</u>		
Manitoba	The pine wood nematode, <u>Bursaphelenchus xylophilus</u> was found in trees declining because of dwarf mistletoe infestation.	Burnes et al. 1983
<u>Pinus contorta</u>		
Rocky Mountains	<u>Dendroctonus ponderosae</u> . The beetles' strong preference for preference for trees of large diameters make it difficult to separate the influence of dwarf mistletoe from that of diameter.	Amman 1978
Colorado	<u>Dendroctonus ponderosae</u> and dwarf mistletoe were associated in all areas evaluated on the White River National Forest.	Lister 1973
Colorado	<u>Dendroctonus ponderosae</u> . In a study of over 1,000 trees little correlation was found between phloem thickness and intensity of dwarf mistletoe infestation.	Hawksworth et al. 1983
Northern Rockies	<u>Dendroctonus ponderosae</u> losses are less in heavy mistletoe-infested stands, probably because of thinner phloem in mistletoe-infested trees. However, an exception is for local bole infections where the thicker bark makes them more likely to be hit by the beetle.	McGregor 1978
Idaho	<u>Dendroctonus ponderosae</u> killed many trees weakened by dwarf mistletoe. In two of three areas studied, beetle losses were more than twice as high in heavily-infested as in healthy or lightly-infested stands.	Parker and Stipe 1974
Idaho	<u>Dendroctonus ponderosae</u> is less likely to attack mistletoe-infested trees because they have thinner phloem - an average of 0.12 inches for diseased trees, and 0.17 inches for healthy trees.	Roe and Amman 1970
General	Interactions of <u>Dendroctonus ponderosae</u> and dwarf mistletoe are complex, and related mostly to ecological status of the stands. Openings caused by mountain pine beetle tend to intensify dwarf mistletoe in the residual stands.	Wellner 1978
Oregon	Large trees that survive <u>Dendroctonus ponderosae</u> epidemics have significantly higher levels of dwarf mistletoe than smaller, less beetle-susceptible trees.	Ziegler 1978
<u>Pinus edulis</u>		
Colorado	<u>Ips</u> sp. and dwarf mistletoe associated with dying pinyon in Mesa Verde National Park.	James and Lister 1978
<u>Pinus jeffreyi</u>		
California	In the Laguna Mountains in southern California pines weakened by dwarf mistletoe are attacked and killed by the California flathead borer, <u>Melanophila californica</u> .	Swain 1972, Swain et al. 1974 Wood et al. 1979
California	In the San Bernardino National Forest an insect-disease complex was responsible for 68% of the tree mortality. The complex consists of 4 insects, 3 fungi, and dwarf mistletoe.	Smith and Roettgering 1982
<u>Pinus ponderosa</u>		
California	About 1/3 of the trees killed in the central Sierra Nevada died from a combination of bark beetles and diseases (including dwarf mistletoe).	California Forest Pest Control Action Council 1975
California	Losses due to bark beetles and dwarf mistletoe were greatly accelerated during the drought years 1977-1978.	Page 1981

Table 2. Reports and observations of dwarf mistletoe-insect associations in relation to tree mortality, 1970-1984.

Host and location	Insect and dwarf mistletoe association	Reference
California	All but 1 of about 200 mistletoe-infected trees killed in campgrounds died from a combination of causes: dwarf mistletoe and insects (bark beetles, engraver beetles, and flathead borers).	Vogler and Scharpf 1981
Arizona	<u>Coloradia pandora</u> . Trees heavily attacked by dwarf mistletoe are more readily killed pandora moth defoliation than lightly- or un-infected trees.	M. Wagner and R. E. Mathiasen, Personal Communication, 1984
Arizona, New Mexico	<u>Ips lecontei</u> . Trees weakened by dwarf mistletoe favor Ips beetle buildup.	Parker 1979
New Mexico	<u>Dendroctonus adjunctus</u> . In three areas studied in the Lincoln National Forest, mortality due to the roundheaded pine beetle was directly related to intensity of dwarf mistletoe infection.	Stevens and Flake 1974
New Mexico	<u>Dendroctonus adjunctus</u> , <u>D. parallecolus</u> , and <u>Ips</u> sp. Found a direct relationships between dwarf mistletoe intensity and bark beetle attack.	Parker et al. 1975
Colorado	<u>Dendroctonus ponderosae</u> . Mountain pine beetle show definite preference for mistletoe-infected trees. Eighty-two % of the beetle killed trees had dwarf mistletoe.	Frye and Landis 1975
Colorado	<u>Dendroctonus ponderosae</u> . Found no association between the bark beetle and dwarf mistletoe where the disease is light, but there may be a strong attraction of the beetle to heavily infected trees.	Johnson et al. 1976
Colorado	<u>Dendroctonus ponderosae</u> . Mountain pine beetle broods and attack density were not significantly different in healthy and heavily mistletoe-infected trees.	McCambridge 1980
Colorado	<u>Dendroctonus ponderosae</u> . Nearly 70% of the trees assumed to have been killed by mountain pine beetle also were attacked by Armillaria root disease and/or dwarf mistletoe.	Fuller, L. R. 1983
Colorado	<u>Dendroctonus ponderosae</u> . In an area in northern Colorado, 31% of the mistletoe-infected trees, but only 20% of the non-mistletoed trees, were killed by the mountain pine beetle.	McCambridge et al. 1982
<u>Pinus radiata</u>		
California	Dwarf mistletoe-infected trees are commonly prone to bark beetle attack.	Old 1979
<u>Pinus</u> spp.		
Guatemala	<u>Dendroctonus frontinalis</u> . Bark beetles are most severe in trees attacked by dwarf mistletoe and a resinous canker.	Anonymous 1973
<u>Pseudotsuga menziesii</u>		
Idaho	<u>Dendroctonus pseudotsugae</u> . No correlation was observed between susceptibility to Douglas-fir beetle and intensity of dwarf mistletoe.	Furniss et al. 1981
California, Arizona	<u>Melanophila drummondi</u> . Trees weakened by Douglas fir dwarf mistletoe are attacked and killed by this borer.	USDA Forest Service 1978; Furniss & Hawksworth, unpublished observations
<u>Juniperus excelsa</u>		
Pakistan	Several species of wood borers are associated with killing junipers infected by dwarf mistletoe.	Chaudrhy and Wali-ur-Rehmin 1979



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# Animal Vectors of Dwarf Mistletoe, with Special Reference to *Arceuthobium americanum* on Lodgepole Pine<sup>1</sup>

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Abstract.--This paper summarizes studies of animal vectors of dwarf mistletoes with special reference to *Arceuthobium americanum* on lodgepole pine in Colorado. A total of 23 vectors has been identified. Results show birds can inoculate susceptible trees and that establishment of dwarf mistletoes beyond the range of their explosive fruits can be explained by vector dissemination of seed.

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## INTRODUCTION

Dwarf mistletoes, *Arceuthobium* spp., are parasitic plants which are regarded as one of the most damaging disease agents of conifers in the United States. An estimated 418 million cubic feet of wood fiber are lost annually because of these pathogens either through growth reduction or tree mortality (Drummond 1982). Dwarf mistletoes also adversely affect wood quality, while reducing cone and seed production in infected trees. An understanding of how dwarf mistletoes are spread is critical to their effective control. Local seed dispersal of dwarf mistletoes via their explosive fruits has been studied in detail (Hinds and Hawksworth 1965), but little is known about the long-range dispersal of *Arceuthobium* spp. The establishment of dwarf mistletoe infections beyond the range of their explosive fruits indicates involvement of vectors and shows the need for further research on this subject. This paper summarizes the evidence currently available on the importance of animal vectors in the spread of dwarf mistletoes, and describes recent research on vectors of *Arceuthobium americanum* in lodgepole pine (*Pinus contorta*) in Colorado.

## LITERATURE REVIEW

Long-distance dissemination of dwarf mistletoes has been a subject of much speculation, but until recently, little definitive research. Isolated pockets of dwarf mistletoe infection have been found beyond the normal range of explosive fruits (table 1). Hawksworth and Weins (1972) reported numerous cases of *A. pusillum* on islands off the Maine Coast and in Lake Michigan. Hawksworth et al. (1975) found *A. cyanocarpum* on ponderosa pine (*P. ponderosa*) 8 km from the nearest infection source, thus suspecting local seed dispersal by birds. Buckland and Marples (1952) believed that scattered *A. campylopodum* infections in regenerated stands of western hemlock (*Tsuga heterophylla*) in British Columbia were initiated by animal or bird activity. Mathiasen (1979), in a survey of the lower peninsula of Michigan, found that *A. pusillum* infected less than one percent of the black spruce stands but occurred in widely scattered infection centers that may have originated from bird-disseminated mistletoe seed. Urban (1968) reported the occurrence of several isolated infection centers of *A. cyanocarpum* in limber pine (*P. flexilis*) stands of Craters of the Moon National Monument, Idaho. He observed that birds and rodents may have been accountable for these infections, which contributed to the distribution and spread of the parasite within the hosts' range. Hudler and Hawksworth (1979), in a study of *A. vaginatum* subsp. *cryptopodum* on ponderosa pine in Colorado, found 32 satellite infection centers ranging in size from one tree to 0.3 ha in a study area of 340 ha. They concluded that some agent, probably birds, was responsible for the occurrence of the infection centers as far as 450 m from the nearest potential seed source.

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Anderson (1949) observed that gray jays (*Perisoreus canadensis*), chickadees (*Parus* spp.) and nuthatches (*Sitta* spp.) may have been responsible for establishing numerous isolated



responsible for establishing numerous isolated infections of A. pusillum in black spruce (Picea mariana) stands in Minnesota. He speculated that seeds could adhere to birds searching for food and that these seeds could later be transferred to healthy trees. In black spruce stands in Minnesota, Ostry (1978) found 12 A. pusillum satellite infection centers that were too far from main infection centers to have originated from explosively disseminated seed. The satellite infection centers ranged in size from a single infected tree to a center 30 by 52 m containing more than 100 infected trees. The centers were located 72 to 736 m away from the main infection center and 30 to 250 m away from the other nearest satellite infection centers. Ostry determined that these satellite infection centers were not remnants of the previous stand but resulted from vector-borne seed.

Hawksworth, Nicholls, and Merrill (unpublished data), in a study of A. americanum on lodgepole pine in Colorado, found 25 isolated infection centers in an area of about 15 ha that appeared to have originated from vector-disseminated mistletoe seed. Isolated pockets of A. douglasii on Douglas-fir (Pseudotsuga menziesii) and A. abietinum on white fir (Abies concolor) were found on the south rim of the Grand Canyon in Arizona. The nearest source of the parasite was 16 km away on the north rim of the Canyon (Hawksworth 1967).

The recent discoveries of isolated populations of A. abietinum in Arizona and

Table 1.--Possible vector initiated dwarf mistletoe sites based upon the literature through 1983.

<u>Arceuthobium</u> species	Location	Reference
<u>A. douglasii</u>	Arizona	Hawksworth 1967
<u>A. abietinum</u>	Arizona	Hawksworth 1967
<u>A. abietinum</u>	Arizona	Mathiasen et al. 1983
<u>A. microcarpum</u>	Arizona	Mathiasen et al. 1983
<u>A. campylopodum</u>	British Columbia	Buckland and Marples 1952
<u>A. cyanocarpum</u>	Colorado	Hawksworth et al. 1975
<u>A. vaginatum</u>	Colorado	Hudler and Hawksworth 1979
<u>A. americanum</u>	Colorado	Authors' observations 1982-83
<u>A. cyanocarpum</u>	Idaho	Urban 1968
<u>A. pusillum</u>	Maine	Hawksworth and Weins 1972
<u>A. pusillum</u>	Michigan	Hawksworth and Weins 1972
<u>A. pusillum</u>	Michigan	Mathiasen 1979
<u>A. pusillum</u>	Minnesota	Anderson 1949
<u>A. pusillum</u>	Minnesota	Ostry 1978

A. microcarpum in New Mexico 270 to 400 km beyond their previously known ranges suggest that long-distance bird dispersal may have been involved (Mathiasen and Jones 1983).

Dwarf mistletoe infections are frequently found in the tops of trees where birds tend to spend time watching and preening (figs. 1A and B). The tops of conifers are highly susceptible because of the high proportion of young susceptible tissues and, once infected, the tops have a greater potential for establishing new infections in surrounding trees (Zilka and Tinnin 1976).

These examples, and others, provide strong circumstantial evidence that vectors are involved in the dissemination of dwarf mistletoe seed and explain the origin of pockets of infection found far from main infection centers. These kind of observations have stimulated studies designed to identify vectors of Arceuthobium spp. in order to fill a major gap in the understanding of dwarf mistletoe life histories (table 2). A summary of research on vectors of dwarf mistletoe follows.

Hudler et al. (1974) found seeds of A. pusillum of black spruce on gray jays and a red squirrel (Tamiasciurus hudsonicus) in a Minnesota study. In some instances, seeds of A. pusillum were known to be carried as far as 100 m by gray jays.

Ostry et al. (1983), in a Minnesota study of A. pusillum on black spruce, captured a total of 250 birds and squirrels representing 45 species. In 2 years of trapping during dwarf mistletoe seed dispersal, they found that 28 animals of seven species had a total of 45 seeds on their bodies. During a peak seed dispersal period in 1974, they found seeds on 12 percent (N=58) of the birds and squirrels captured and, in 1975, they found seeds on 20 percent (N=64) of the animals captured. Animals found with seed were the gray jay, yellow warbler (Dendroica petechia), palm warbler (Dendroica palmarum), yellow-rumped warbler (Dendroica coronata), dark-eyed junco (Junco hyemalis), red squirrel, and flying squirrel (Glaucomys sabrinus). Because of the difficulty of observing animals actually inoculating trees in the field, Ostry et al. (1983) set up controlled experiments in which dwarf mistletoe seeds were placed on captive birds who were observed in cages with potted black spruce. Birds were observed removing some of the seeds during preening. Several of the removed seeds stuck to susceptible portions of trees, providing the first conclusive evidence that birds can inoculate trees. Presumably this is what also occurs under field conditions, according to the authors.

Singh (1982) found that A. pusillum is one of the principal agents damaging black spruce on the island of Newfoundland, where it causes up to 86-percent tree infection and up to 37-percent tree mortality. The patchy distribution of the parasite matched sites inhabited by the

Newfoundland gray jay (Perisoreus canadensis sanfordi), some of which harbored dwarf mistletoe seeds.

Zilka and Tinnin (1976) studied bird-dwarf mistletoe relationships in the Pacific Northwest. During a brief collection period in the fall of 1973, 18 birds representing 11 species were collected. Four birds, Cassin's finch (Carpodacus cassinii), mountain chickadee (Parus gambeli), red crossbill (Loxia curvirostra), and Steller's jay (Cyanocitta stelleri) carried seeds on their feathers. The authors reported that the Steller's jay is particularly important as a potential vector because it often spends time in the highest perches of a tree watching and preening. In addition, most of the species they observed frequently chose brooms for night perches where they would easily be hit by dispersing seed. The authors reported 28 instances, in 350 hrs of observation, of birds being struck by seeds.



Figure 1A.--Gray jay on a typical preening or watching perch at the top of a host tree.



Figure 1B.--Dwarf mistletoe infection typical of those from vector-disseminated seed, on top of a Douglas fir tree.

Table 2.--Number of dwarf mistletoe vector species identified by various investigators.

No. vector species identified	Location	Mistletoe in study area	Reference
4	Oregon	6 <u>Arceuthobium</u> spp.	Zilka and Tinnin 1976
1	Oregon	4 <u>Arceuthobium</u> spp.	Lemons 1978
2	Minnesota	<u>Arceuthobium pusillum</u>	Hudler et al. 1974
7	Minnesota	<u>Arceuthobium pusillum</u>	Ostry et al. 1983
1	Newfoundland	<u>Arceuthobium pusillum</u>	Singh 1982
5	Colorado	<u>Arceuthobium vaginatum</u>	Hudler and Hawksworth 1979
14	Colorado	<u>Arceuthobium americanum</u>	Authors' observations 1982-83

Many bird species use mosses and foliose lichens for nesting materials that also serve as an excellent reservoir for viable dwarf mistletoe seeds. Zilka and Tinnin (1976) reported finding seeds in five of seven nests observed; these seeds could constitute an important source of inoculum in regrowth of clear-cut areas. They also identified 10 avian species where frequency of occurrence and behavior imply a significant role in seed transport.

Hudler and Hawksworth (1979) conducted studies in a Colorado ponderosa pine forest infested with A. vaginatum subsp. cryptopodum. A total of 411 birds representing 21 species were trapped, with 4 percent carrying dwarf mistletoe seed. These birds included the pygmy nuthatch (Sitta pygmaea), mountain chickadee, gray-headed junco (Junco caniceps), chipping sparrow (Spizella passerina), and Williamson's sapsucker (Sphyrapicus thyroideus). On eight occasions they observed mountain chickadees to be struck by discharging seeds which adhered to their feathers as was the case with pygmy nuthatches on six occasions. In five instances, chickadees hit by discharged seeds continued foraging in tips of ponderosa pine branches, and seeds were transferred from birds to pine needles. These observations confirmed that birds can inoculate susceptible trees in the field.

Lemons (1978) investigated the role that small mammals play in dwarf mistletoe seed dispersal in the Malheur National Forest in Oregon. Arceuthobium spp. present were A. campylopodum, A. douglasii, A. laricis and A. americanum, and the primary host species of each were Pinus ponderosa, Pseudotsuga menziesii, Larix occidentalis and Pinus contorta,



respectively. Two study areas were established. Red squirrels were examined in each area when the seeds began to disperse. No seeds were found on squirrels where infection was low, but 50 percent of the squirrels trapped or shot in a more heavily infected area carried seeds on their fur. Lemons thought that squirrels carried mistletoe seed over distances up to 150 m, but doubted that many infections resulted because most seeds carried on the fur were probably groomed off in an uninfected part of the host tree.

Another association that may lead to mistletoe seed vectoring is the establishment of nests by birds and mammals in dwarf mistletoe-caused witches' brooms (Tinnin et al. 1982; Weir 1916). Some records and observations of such nestings are given in table 3. Although, in general, most nesting activities are completed before late summer-early fall mistletoe seed dispersal, late nesting by some species overlaps the seed dispersal period.

Based on the results of foregoing studies, 23 bird and mammal vectors have been identified (table 4). Vectors of dwarf mistletoe may be

Table 4.--Identified vectors of dwarf mistletoe based upon the literature through 1983.

Birds	Mammals
Gray Jay ( <u>Perisoreus canadensis</u> )	Red Squirrel ( <u>Tamiasciurus hudsonicus</u> )
Steller's Jay ( <u>Cyanocitta stelleri</u> )	Flying Squirrel ( <u>Glaucomys sabrinus</u> )
Three-toed Woodpecker ( <u>Picoides tridactylus</u> )	Golden-mantled Squirrel ( <u>Citellus lateralis</u> )
Williamson's Sapsucker ( <u>Sphyrapicus thyroideus</u> )	Pine Marten ( <u>Martes americana</u> )
Robin ( <u>Turdus migratorius</u> )	Least Chipmunk ( <u>Eutamias minimus</u> )
Dark-eyed Junco ( <u>Junco hyemalis</u> )	
Gray-headed Junco ( <u>Junco caniceps</u> )	
Hermit Thrush ( <u>Catharus guttatus</u> )	
N. Saw-whet Owl ( <u>Aegolius acadicus</u> )	
Yellow Warbler ( <u>Dendroica petechia</u> )	
Palm Warbler ( <u>Dendroica palmarum</u> )	
Yellow-rumped Warbler ( <u>Dendroica coronata</u> )	
Mountain Chickadee ( <u>Parus gambelli</u> )	
Cassin's Finch ( <u>Carpodacus cassinii</u> )	
Pygmy Nuthatch ( <u>Sitta pygmaea</u> )	
Chipping Sparrow ( <u>Spizella passerina</u> )	
Townsend's Solitaire ( <u>Myadestes townsendi</u> )	
Red Crossbill ( <u>Loxia curvirostra</u> )	

more important in the short-and long-range distribution of dwarf mistletoe than originally thought. Even though animal dissemination and resulting infection may be infrequent, this probable means of seed dispersal enables dwarf mistletoe to become established and to spread in new locations. These new infection centers may hinder otherwise effective silvicultural control measures.

#### LOGEPOLE PINE DWARF MISTLETOE STUDY

To gain more insight into dwarf mistletoe-vector biology, we conducted a study to identify vectors of lodgepole pine dwarf mistletoe in Colorado and to determine their potential importance in the establishment of new infection centers.

Table 3.--Reports of birds and mammals nesting in Arceuthobium-caused witches' brooms.

Animal	Location	Reference
BIRDS		
Pine Siskin ( <u>Carduelis pinus</u> )	Oregon	Zilka 1973
Gray Jay ( <u>Perisoreus canadensis</u> )	Minnesota	Warren 1899
Red Crossbill ( <u>Loxia curvirostra</u> )	Colorado	Bailey et al. 1953
Robin ( <u>Turdus migratorius</u> )	Colorado	Authors' observation
House Wren ( <u>Troglodytes aedon</u> )	Arizona	Authors' observation
Spotted Owl ( <u>Strix occidentalis</u> )	New Mexico	Lignon 1926
Accipiter Hawks ( <u>Accipiter spp.</u> )	Oregon	Forsman 1983
		Reynolds et al. 1982
MAMMALS		
Red Squirrel ( <u>Tamiasciurus hudsonicus</u> )	Minnesota	Ostry 1978
	Colorado	Hatt 1943
Abert Squirrel ( <u>Sciurus aberti</u> )	Colorado	Farentinos 1972

## METHODS

Our study was conducted in 1982-1983 during A. americanum seed dispersal on the Fraser Experimental Forest in the Arapaho National Forest near Winter Park, Colorado. The lodgepole pine forest is mainly Abies lasiocarpa/Vaccinium scoparium habitat type. Birds and mammals were trapped in lodgepole pine stands using cell traps and mist nets up to 445 m in total length (fig. 2). Trapped animals were examined; and the number and location of seeds adhering to their bodies were observed and recorded (fig. 3). The seeds were removed and some were tested for viability by immersing them in 0.2 percent W/V aqueous 2, 3, 5-triphenyl tetrazolium chloride (TTC) for 24 to 48 hr. The viable seed endosperm stained pink while nonviable seeds remained unstained (Scharpf 1970). Birds were then banded and mammals were ear-tagged so that individuals could be identified upon recapture. Movements of animals within the study area were monitored by retrapping, color marking, or radio telemetry (fig. 4).

Timing of dwarf mistletoe seed dispersal throughout August and September was monitored by making daily seed counts on cloth seed traps placed on the ground near infected trees. These seeds were also checked for viability using the TTC chemical test. The distribution of satellite infection centers on the study area was determined by surveying for the presence or absence of dwarf mistletoe on 1,450 plots taken at 1-chain intervals in a 60-ha area.



Figure 2.--Steller's jay caught in a mist net used to trap birds.



Figure 3.--Examining a Steller's jay carefully for mistletoe seed or, Steller's jay examining pathologist for seeds.



Figure 4.--Gray jay with a 3.5 g radio transmitter used to follow its movements between dwarf mistletoe infected and healthy lodgepole pine stands.

## RESULTS

Ten bird (table 5) and four mammal (table 6) species were identified as potential vectors of dwarf mistletoe. A total of 626 birds (including retraps = IRT) of 30 species and 300 mammals (IRT) of four species was trapped and examined for seed. For the entire study period, 7 percent of the birds and 9 percent of the mammals carried



seed (table 7). A total of 84 seeds was found on 71 animals during this period (44 on birds and 27 on mammals). During a 16-day peak seed-dispersal period in 1982, 22 percent of the birds (N = 55 IRT) and 20 percent of the mammals (N = 80 IRT) had seed. The most important vectors were the gray jay, Steller's jay (fig. 5), mountain chickadee, and least chipmunk (*Eutamias minimus*) (fig 6).

Five gray jays were radio-tracked in 1983, verifying that the birds frequently moved back and forth between infected and healthy stands of lodgepole pine. Some of the radioed birds were known to be carrying seed at the time they were radio-tracked. Color-marked birds provided similar information. Telemetry studies of five gray jays showed that their home ranges covered 21 to 51 ha.

None of the birds were observed eating mistletoe seed. They acquired seed, however, when foraging for food in infected trees as seeds were being explosively discharged from dwarf mistletoe fruits. The seeds, sticky with viscin, easily stuck to feathers. Most seeds were found on lower body parts around legs, on the breast, and under wings and tail. Of 20 seeds removed from animals and tested with TTC, 65 percent were viable.

Both resident and migratory birds were captured with seeds on their feathers. This indicates that seeds could be disseminated over short distances by resident birds and over long distances by migratory birds.

Table 5.--Bird vectors of lodgepole pine dwarf mistletoe trapped on the Fraser Experimental Forest in Colorado during 1982-1983.

Species	1982		1983	
	No. Trapped	% with seed	No. Trapped	% with seed
Steller's Jay ( <i>Cyanocitta stelleri</i> )	10	30	14	7
Gray Jay ( <i>Perisoreus canadensis</i> )	41	24	74	15
Three-Toed Woodpecker ( <i>Picoides tridactylus</i> )	8	13	1	0
Mt. Chickadee ( <i>Parus gambeli</i> )	23	9	52	6
Y.-Rumped Warbler ( <i>Dendroica coronata</i> )	57	5	9	0
Robin ( <i>Turdus migratorius</i> )	21	5	11	9
G.-Headed Junco ( <i>Junco caniceps</i> )	39	3	62	3
Hermit Thrush ( <i>Catharus guttatus</i> )	33	3	39	3
Townsend's Solitaire ( <i>Myadestes townsendi</i> )	1	0	2	50
N. Saw-whet Owl ( <i>Aegolius acadicus</i> )	4	0	5	40

Table 6.--Mammal vectors of lodgepole pine dwarf mistletoe trapped on the Fraser Experimental Forest in Colorado during 1982-1983.

Species	1982		1983	
	No. Trapped	% with seed	No. Trapped	% with seed
Least Chipmunk ( <i>Eutamias minimus</i> )	78	19	187	4
G.-mantled Squirrel ( <i>Citellus lateralis</i> )	17	12	3	33
Pine Marten ( <i>Martes americana</i> )	1	100	0	0
Red Squirrel ( <i>Tamiasciurus hudsonicus</i> )	0	0	9	11

Table 7.--Summary of vectors of lodgepole pine dwarf mistletoe, Fraser Experimental Forest, Colorado, 1982-1983.

Animal	No. trapped	No. with seed	% with seed
Birds	626	44	7
Mammals	300	27	9
Totals	926 <sup>1</sup>	71 <sup>2</sup>	$\bar{X} = 7.2$

<sup>1</sup>Includes retrapped animals.

<sup>2</sup>A total of 84 seeds was found on these 71 animals.

One mammal, a least chipmunk, was seen feeding on dwarf mistletoe berries for about 20 minutes. In the process, it ended up with at least five seeds on its fur. Least chipmunks spent most of their time on the ground where they inadvertently picked up seeds already released from berries. This was also true for the golden-mantled squirrel (*Citellus lateralis*). In contrast, red squirrels also spent time in trees cutting down cones and storing mushrooms in dwarf mistletoe brooms and other branches. They were frequently observed brushing against mistletoe shoots during these activities. The home ranges of these mammals were small and appeared to be important only in local dwarf mistletoe dissemination and intensification.

During the 1983 study period, a total of 966 seeds was counted on seed traps at three different elevations. TTC tests revealed that 65 percent of a sample of these seeds (N = 66) were viable. Beginning the week of August 15, dispersal progressed from the lowest elevation to highest elevation over a period of 6 weeks (fig. 7). This increased the time of potential seed contact for migratory birds and resident birds that had the same kind of elevation differences in their home range as did the gray and Steller's

jays. Because of this longer seed dispersal period, there were more opportunities for these birds to come in contact with seeds than in infected stands with little elevational differences and a corresponding shorter seed dispersal period.

A total of 1,450 plots (established at 20-m grids) was evaluated in a 60-ha, 70-year-old lodgepole pine stand to determine presence or absence of dwarf mistletoe and to identify satellite infection centers that may have originated from vector-carried seed. Of 1,450 plots, 218 were located in a non-lodgepole pine type. Of the remaining 1,232 plots, 871 were infested with dwarf mistletoe. Twenty-five satellite infection centers were found within the lodgepole type that otherwise had no mistletoe, or about 1.7 centers/ha. The number of infected trees in these satellite areas ranged from one to 10 trees (mean 3.5). Most of these areas were positioned along the edges of old logging roads or other forest openings favored by birds. All 25 satellite infection centers were too far removed from main infection centers, or too protected by healthy trees to have originated from seeds expelled from mistletoe fruits alone. Distances from main infection centers ranged from 12 to 65 (ave. 27) m. This portion of the study is continuing and the results will be described in more detail in a subsequent paper.

#### DISCUSSION

As outlined in this paper, there are several reports of dwarf mistletoe infection centers that originated from other than explosively discharged seeds and that are not remnants of fire escapes. These observations have served as the basis for much speculation that vectors are responsible for disseminating dwarf mistletoe seeds. Dissemination by vectors would explain the origin of satellite infection centers far removed from main infection centers. The vector idea is logical because the sticky seeds are expelled



Figure 5.--Dwarf mistletoe seed sticking to the tail feather of a Steller's jay.



Figure 6.--Dwarf mistletoe seed sticking to the tail of a least chipmunk.

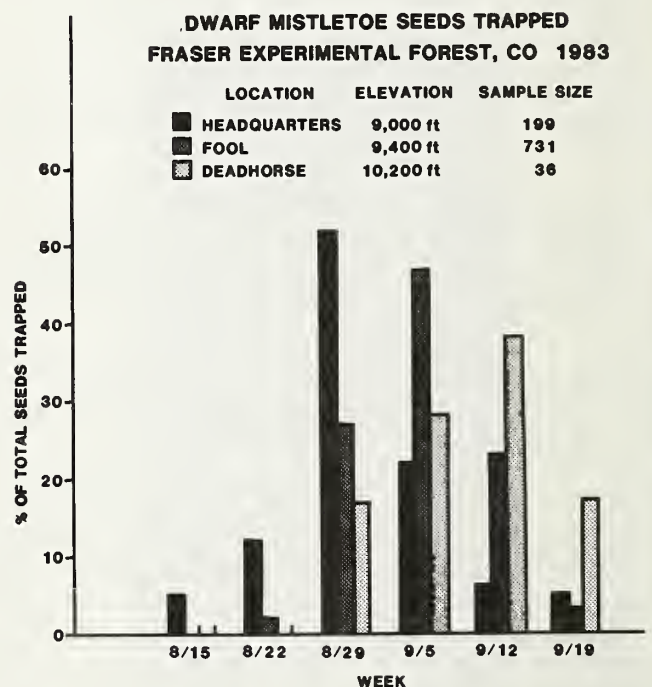


Figure 7.--Number of dwarf mistletoe seeds trapped at three elevations on the Fraser Experimental Forest, Colorado, during 1983.

from fruits at initial velocities of about 27 m per second for distances up to 15 m (Hawksworth and Weins 1972) and will generally stick to anything they hit, including animals. Animals can come in frequent contact with seed when foraging for insects, storing food, and nesting in mistletoe brooms. Further, dwarf mistletoe seeds removed during the animals' regular grooming can cause infection if the seeds are viable and deposited in host trees where infections can occur.



For animals to transport mistletoe seeds that cause infection, certain criteria must be met (Zilka and Tinnin 1976): (1) the seed must become attached to the animal; (2) female seeds must land close enough to a male infection to facilitate pollination and establish seed production because dwarf mistletoe plants are dioecious<sup>3</sup>; (3) the seed must arrive in viable condition on a susceptible host; (4) the seed must come in contact with susceptible parts of the host for infection to occur. Because of these specific requirements, vector-disseminated mistletoe seed infections are probably infrequent, but scattered cumulative infections that develop over a period of time could occur frequently enough to be of concern in the effective control of this parasite.

Our study showed that 22 percent of the birds carried dwarf mistletoe seed during the peak seed dispersal period of *A. americanum*. Therefore, in a 1,000-bird population, 220 could be carrying seeds at any one time. Several seeds carried by this number of birds could meet all of the above criteria and establish new infections in otherwise healthy stands.

Our study, as well as the other studies reviewed in this paper, has clearly documented that animals can vector dwarf mistletoe seeds and at least two studies showed that birds can inoculate susceptible trees. The movements and behavior of the different vector species play a key role in how and where dwarf mistletoe seed is transported. In general most birds do not eat mistletoe seeds or, if they do, the seeds are not passed through the digestive track in viable condition (Hudler and Hawksworth 1979; Zilka and Tinnin 1976; Ostry et al. 1983). All the reviewed studies show that viable dwarf mistletoe seeds are generally transported on the external surfaces of animals.

The distance seeds can be transported by animals depends upon how large the animals' home range is and whether they are migratory or resident. It is generally believed that mammal vectors are only important in local spread and intensification because of their relatively small home ranges. Resident birds, such as the gray and Steller's jays, mountain chickadees, and gray-headed juncos, are most important in local and short-distance spread of dwarf mistletoe. In our study the gray jay was the most important vector as in the Minnesota studies (Hudler et al. 1974; Ostry et al. 1983).

Migratory birds, such as the warblers (*Dendroica* spp.), robins (*Turdus migratorius*), and hermit thrushes (*Catharus guttatus*), may be more important in long-distance spread of dwarf mistletoes. Many dwarf mistletoe species release seed during the time when some bird species are migrating southward in late summer and fall. Due to habitat specificity, warblers selecting a lodgepole pine habitat in one area will probably choose the same habitat in other areas during migration, if available. During migration, seeds picked up in an infected lodgepole pine stand could be carried and deposited in a lodgepole pine stand some distance away from where the seeds were originally acquired. This habitat specificity is important and increases the chance that a successful infection will result because dwarf mistletoes are generally host specific (Hawksworth and Weins 1972).

#### CONCLUSION

Several recent studies of vector-dwarf mistletoe relationships have provided definitive documentation that animals do serve as vectors of dwarf mistletoe seeds and that they can inoculate infectable parts of susceptible hosts. Some of the studies show that vectors may be more important in the spread of the parasite than originally thought.

Highly specific requirements must be satisfied before a successful dwarf mistletoe infection can occur. Once infection occurs, several years must pass before the life cycle of this pathogen can be completed to establish an infection center. Although infections resulting from vector-disseminated seed are probably infrequent because of these specific requirements, the cumulative establishment of scattered infection centers over a long period of time and, subsequent local dispersal, can accelerate and intensify the spread of dwarf mistletoes. Once established, each center of dwarf mistletoe infection will remain an inoculum reservoir for future infections unless controlled by silvicultural methods. Knowing where and how to find satellite infection centers that originate from vector-borne seed will enable forest managers to control these areas while they are still small enough to protect commercial stands. Thus, an understanding of the role animals play in the dispersal of dwarf mistletoe seed is essential for the effective control of this damaging forest disease.

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<sup>3</sup>The effective distance of pollen dispersal in the dwarf mistletoes has not been determined, but pollen dispersal distances for *A. americanum* of 150 m have been reported in Colorado (Penfield et al. 1976) and dispersal distances of 400 m have been reported in Manitoba (Gilbert and Punter 1984).

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# Relationships between Dwarf Mistletoes and Habitat Types in Western Coniferous Forests<sup>1</sup>

Robert L. Mathiasen and Elizabeth A. Blake<sup>2</sup>

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Abstract.--Past studies of the relationship between habitat types and the incidence and distribution of dwarf mistletoes are reviewed. Recent productivity studies of several mixed conifer species in relation to habitat type and dwarf mistletoe infection are examined.

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## INTRODUCTION

During the last decade the use of habitat types to classify forest vegetation (Daubenmire 1952, Daubenmire and Daubenmire 1968) has been expanded to most regions of the western United States (Pfister 1976, Pfister and Arno 1980). A habitat type is usually defined as a unit of land capable of producing similar plant communities at climax (Daubenmire 1968). The habitat type concept is based on the climax stage of succession as defined by Tansley (1935), and reflects an integration of environmental factors that affect forest vegetation. Although a single habitat type may support several seral plant communities, the final stage of plant succession within the habitat type will be a specific climax community. Therefore, habitat type classification systems use climax plant communities as an integrated indicator of environmental conditions that influence plant reproduction, competition, and community development (Steele et al. 1981).

The name of each habitat type is based on the climax community type. The first part of the name is based on the climax tree species and the second part is based on the dominant undergrowth species. Hence the habitat type Pinus ponderosa/Muhlenbergia virescens (Ponderosa pine/screwleaf muhly) is dominated by a ponderosa pine overstory with the ground cover dominated by screwleaf muhly. A series encompasses all habitat types that have the same dominant tree species at climax. Phases of habitat types represent variations in climax plant communities which are less distinct than those between habitat types. Phases may also represent variants of habitat types that are persistent late seral communities (Hanks et al. 1983).

The use of a habitat type classification system does not require the presence of climax vegetation to classify forest land. Habitat types

can usually be recognized during various stages of plant succession by comparing reproduction of tree species present in an area with known successional patterns of a habitat type, and by examination of the dominant undergrowth species present.

Ecological and management implications of habitat types have been expressed in various terms beginning with Daubenmire's 1952 publication. Ecologically, habitat type systems provide a great deal of information concerning the distribution of species for given habitat types, successional patterns and relationships, and the environmental factors influencing these relationships. Resource managers have been developing management implications based on habitat types for timber, range, watershed, and recreation resources (Layser 1974). In addition, application of habitat type systems in research can provide a means of improving the identification of research needs, the design of sampling techniques, and the reporting of results (Layser 1974, Daubenmire 1976).

## DWARF MISTLETOES AND HABITAT TYPES

Now let us turn our attention to the relationships between the incidence and distribution of the dwarf mistletoes (Arceuthobium spp.) and vegetation types or habitat types. As early as 1929, Dowding reported that lodgepole pine dwarf mistletoe (Arceuthobium americanum Nutt. ex Engelm.) was more abundant in jack pine (Pinus banksiana Lamb.) stands of the "pine-moss" type than in those of the "pine-heath" type in central Alberta. Her conclusions were based primarily on observations of several jack pine stands representing these vegetation types. The first (and still the most explicit) report of a distinct relationship between the distribution of a dwarf mistletoe and habitat types was made by Daubenmire (1961). He reported that infection by Arceuthobium campylopodum Engelm. on ponderosa pine (Pinus ponderosa Laws.) was common in the Pinus ponderosa/Agropyron spicatum and Pinus ponderosa/Purshia tridentata habitat types. However, infection was not found in five other ponderosa pine habitat types he recognized in eastern Washington and northern Idaho. The habitat types infested with dwarf mistletoe represented the driest and poorest sites for ponderosa pine

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in that region in terms of tree growth and soil texture. Daubenmire and Daubenmire (1968) reported the occurrence of *A. campylopodum* in the *Pinus ponderosa*/*Festuca idahoensis* and *Pinus ponderosa*/*Stipa comata* habitat types in addition to the two habitat types previously cited (Daubenmire 1961). Daubenmire postulated that ponderosa pine was susceptible to dwarf mistletoe only where it was associated with xerophytic grasses and *Purshia*, possibly because the pine grew slowly in these habitat types (Daubenmire and Daubenmire 1968, Daubenmire 1969a, 1969b). Schlatterer (1972) also reported that *A. campylopodum* was most prevalent in habitat types representing low productivity classes for ponderosa pine in central Idaho. In addition, Schlatterer reported a similar relationship between Douglas-fir dwarf mistletoe (*Arceuthobium douglasii* Engelm.) and habitat types he recognized in his Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) series in central Idaho. Dwarf mistletoe incidence and impact was most severe in the habitat type with the poorest productivity potential for Douglas-fir (*Pseudotsuga menziesii*/*Symphoricarpos oreophilus*) and least severe in those habitat types with high productivity (*Pseudotsuga menziesii*/*Calamagrostis rubescens* and *Pseudotsuga menziesii*/Tall Shrubs). In the *Pseudotsuga menziesii*/*Carex geyeri* - *Symphoricarpos oreophilus* habitat type dwarf mistletoe was common on the sites representing poor productivity for Douglas-fir and absent from the high productivity sites. Schlatterer hypothesized that low incidence of Douglas-fir dwarf mistletoe was related to the faster growth rate of Douglas-fir in the highly productive habitat types.

Roe and Amman (1970) collected data on the incidence of *Arceuthobium americanum* in 42 lodgepole pine (*Pinus contorta* Doug. ex Loud.) stands in southeastern Idaho and western Wyoming. Three habitat types were represented in the study: *Abies lasiocarpa*/*Vaccinium scoparium* (ABLA/VASC), *Abies lasiocarpa*/*Pachystima myrsinites* (ABLA/PAMY), and *Pseudotsuga menziesii*/*Calamagrostis rubescens* (PSME/CARU). The relative intensity indexes<sup>3</sup> for dwarf mistletoe were 2.5 for the ABLA/VASC habitat type, 1.9 for the ABLA/PAMY habitat type and 1.6 for the PSME/CARU habitat type. Roe and Amman did not speculate on the possible relationships their data may have demonstrated for dwarf mistletoe distribution by habitat types.

Fuller and Hofstetler (1980) reported percent infection and average dwarf mistletoe ratings for lodgepole pine infested by *A. americanum* by understory vegetation (but not habitat types) based on 277 plots located throughout Colorado. These preliminary results indicate relatively large differences for the frequency and severity of dwarf mistletoe between stands dominated by different understory vegetation (Table 1). The data could be stratified by habitat types now that habitat type classifications are available for most of Colorado.

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Based on a 4 class intensity scale: (1) no infection, (2) less than 1/3 of trees infected, (3) 1/3 to 2/3 of trees infected and (4) more than 2/3 of trees infected.

Table 1.--Lodgepole pine dwarf mistletoe frequency and average mistletoe rating by understory vegetation based on 277 plots from Colorado (from Fuller and Hofstetler 1980).

Predominant Understory Vegetation	% of Plots With Dwarf Mistletoe	Average Mistletoe Rating <sup>1</sup>
<i>Shepherdia canadensis</i>	50	1.3
<i>Vaccinium scoparium</i>	30	1.1
<i>Juniperus communis</i>	35	1.1
<i>Arctostaphylos uva-ursi</i>	23	1.0
<i>Arnica cordifolia</i>	33	1.0
Grasses (no shrubs)	20	0.7

<sup>1</sup> Based on the 6-class dwarf mistletoe rating system, Hawksworth 1977.

The recent findings of Merrill (1983) support the contention that dwarf mistletoes are most prevalent and severe in habitat types that represent xeric or poor sites for their principal host. Her extensive surveys in Colorado demonstrated that the occurrence of *Arceuthobium vaginatum* subsp. *cryptopodum* (Engelm.) Hawksw. & Wiens was significantly more frequent in the *Pinus ponderosa*/*Muhlenbergia montana* (PIPO/MUMO) habitat type than in the seven other habitat types sampled (Figure 1). The PIPO/MUMO habitat type represents one of the most xeric habitat types of the ponderosa pine series in Colorado (Hess 1981). In addition, the severity of dwarf mistletoe infection, as indicated by mean dwarf mistletoe ratings based on the 6-class system (Hawksworth 1977), was highest in the PIPO/MUMO habitat type. The mean dwarf mistletoe rating of this habitat type was significantly higher than five of the habitat types, but not significantly higher than two of the habitat types she sampled.

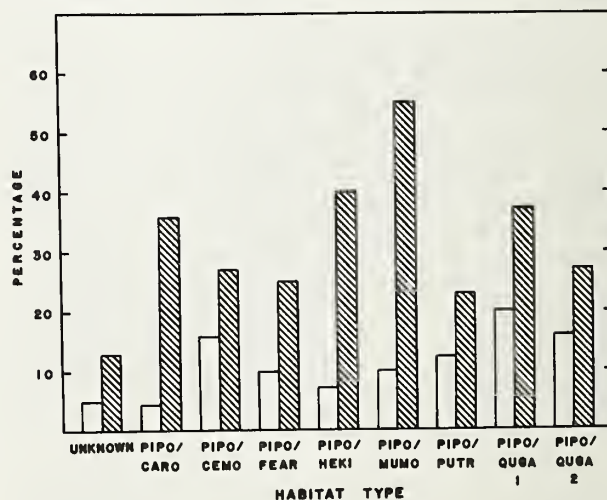


Figure 1.--Relationship between incidence of ponderosa pine dwarf mistletoe and habitat type on 547 plots in Colorado. The open bars show the percent distribution of plots by habitat types and the cross-hatched bars show the percent of plots with dwarf mistletoe in each habitat type (from Merrill 1983).



Hawksworth (1959, 1961, 1969, 1975) has considered many of the ecological factors that could influence the distribution of dwarf mistletoes. These factors include stand history, temperature, site quality, elevation, topography, and aspect. There appear to be fairly well defined relationships between some dwarf mistletoes, the elevational ranges of their principal hosts, and topographic features such as slope steepness, slope position, and aspect (Hawksworth 1969). Merrill (1983) examined these factors in relation to those habitat types with the highest incidence and severity of *A. vaginatum* subsp. *cryptopodum*. She found a high degree of correlation between habitat types, topographic features, and elevational positions and high incidence of dwarf mistletoe.

Much of the information available concerning the relationships between dwarf mistletoes and habitat types is found in the many habitat type classifications completed in the western United States. Several classifications have provided only qualitative data, reporting distribution and incidence of dwarf mistletoes by habitat types as low, minor, present, uncommon, moderate, high, damaging, severe, prevalent, or significant (Hall 1973, Vol-land 1982, Henderson et al. 1977, Hopkins 1979a, 1979b, Hanks et al. 1983). For example, Hanks et al. (1983) presented the percentage of stands sampled with light to heavy infection by *A. vaginatum* subsp. *cryptopodum* for a single ponderosa pine habitat type in northern Arizona. Incidence of mistletoe in their remaining ponderosa pine habitat types were reported as a qualitative rating from light to heavy (Table 2). Another classification has rated habitat types as either highly susceptible or resistant to mistletoe infection (Schlatterer 1972), but this use of terminology is misleading.

Only a few habitat type classifications have attempted to provide quantitative data on the incidence of dwarf mistletoes for different habitat types. Daubenmire and Daubenmire (1968) listed the number of stands they sampled where *A. campylopodum* was present for each habitat type in their ponderosa pine series. Franklin and Dyrness (1973) provided constancy and coverage figures for *A. campylopodum* in habitat types based on Daubenmire and Daubenmire's (1968) data. Pfister et al. (1972) reported percentages of plots sampled by habitat type that had Douglas-fir or larch mistletoe (*Arceuthobium douglasii* or *A. laricis* (Piper) St. John) present in light, moderate, or severe levels (Table 3). However, they did not include this information in their final habitat type classification for western Montana (Pfister et al. 1977).

If it appears we have little information regarding the distribution of dwarf mistletoes by habitat types, we have even less information on the growth loss and mortality caused by these parasites in different habitat types. Moir and Ludwig (1979) reported that *A. abietinum* Engelm. ex Munz reduces height growth of white fir (*Abies concolor* (Gord. & Glend.) Lindl.) in the *Abies concolor*-*Pseudotsuga menziesii*/Sparse Understory habitat type on the Kaibab Plateau. However they did not provide quantitative data to support their contention. Ziegler (1978) reported significant

Table 2. Incidence of *Arceuthobium vaginatum* subsp. *cryptopodum* by ponderosa pine habitat types in northern Arizona (from Hanks et al. 1983).

Habitat Type <sup>1</sup>	Incidence of Dwarf Mistletoe
PIPO/MUVI; Typical Phase	Damage light to moderate in 77% of stands
PIPO/MUVI; QUGA Phase	Light to moderate mistletoe in 70% of stands
PIPO/MUVI-FEAR; Typical Phase	Light, moderate, heavy levels in equal frequencies
PIPO/MUVI-FEAR; QUGA Phase	Light to moderate mistletoe
PIPO/FEAR; Typical Phase	Present in moderate to heavy amounts
PIPO/FEAR; QUGA Phase	Moderate to heavy mistletoe
PIPO/FEAR; BOGR Phase	Moderate to heavy mistletoe
PIPO/BOGR; Typical Phase	Light to moderate mistletoe
PIPO/BOGR; PIED Phase	No mistletoe data given
PIPO/BOGR; QUGA Phase	Mistletoe incidence light
PIPO/BOGR; ARTR Phase	Light to heavy mistletoe

<sup>1</sup>See Hanks et al. (1983) for meaning of habitat type abbreviations.

toe-infected lodgepole pines in some habitat types, but not in others, in the vicinity of Crater Lake, Oregon. He also demonstrated a significant reduction in five-year basal area increment with increasing levels of mistletoe infection in the *Pinus contorta*/*Carex-Lupinus* community. However, heavy mistletoe infection did not affect sapwood thickness in lodgepole pine in any of the habitat types sampled.

#### RELATIONSHIPS OF DOUGLAS-FIR DWARF MISTLETOE WITH HABITAT TYPES IN THE SOUTHWEST

We compared ten-year periodic annual increment for dominant and codominant Douglas-firs between nine southwestern habitat types (Mathiasen and Blake 1984). Data were collected from spruce-fir and mixed conifer stands in Arizona and New Mexico from 1979 to 1981. Diameter at breast height (DBH, nearest 0.1 inch), DMR (six-class system, Hawksworth 1977), crown class (dominant, codominant, intermediate, suppressed), and five- and ten-year radial growth at DBH were recorded for a total of 27,000 Douglas-fir trees.

Ten year periodic annual increment (PAI) for dominant, codominant, and intermediate Douglas-firs was significantly different at  $p = 0.10$  in all the habitat types studied regardless of DMR class. Heavy Douglas-fir dwarf mistletoe infec-

Table 3.--Observance of Douglas-fir (DF) and larch (WL)  
dwarf mistletoe for twelve habitat types in western  
Montana (from Pfister et al. 1972).

Habitat Type <sup>1</sup>	Stands Sampled N	Stands w/DF n	Douglas-fir				Stands w/WL n	Western Larch			
			None	Light	Moderate	Severe		None	Light	Moderate	Severe
			Percent					Percent			
PSME/CARU	15	15	60	13	13	13	2	0	0	0	0
PSME/ARUV	10	10	60	20	10	10	3	0	0	0	0
PSME/VACA	15	15	80	20	0	0	14	64	14	7	14
PSME/SYAL	23	23	52	22	22	4	5	75	0	25	0
PSME/PHMA	25	25	56	24	8	12	7	86	0	14	0
PSME/XETE	15	15	53	27	7	7	7	100	0	0	0
ABGR/CLUN	10	10	80	10	10	0	7	86	0	0	14
ABLA/CLUN	17	15	60	7	27	7	11	27	0	27	45
ABLA/XETE-CARU	9	8	88	0	0	12	5	100	0	0	0
ABLA/CLUN-VACA	7	6	100	0	0	0	5	100	0	0	0
THPL/CLUN	8	8	63	12	12	12	8	25	25	25	25
TSHE/CLUN	7	5	100	0	0	0	6	17	17	50	17

<sup>1</sup>See Pfister et al. (1977) for meaning of habitat type abbreviations.

tion significantly reduced ten-year PAI in all the habitats. Moderate infection significantly reduced growth in the *Picea pungens*-*Picea engelmannii*/Erigeron superbus and *Abies concolor*-*Pseudotsuga menziesii*/Sparse Understory; *Robinia neomexicana* Phase habitat types. In addition, the percentages of growth reduction (cubic feet/acre/year) for moderate and heavily infected Douglas-firs varied greatly between habitat types (Table 4). A potential damage rating representing damage due to dwarf mistletoe was assigned to each habitat type by adding percent growth reduction for moderately (DMR class 3-4) and heavily (DMR class 5-6) infected trees.

Table 4. Percent growth reduction for dominant and codominant Douglas-firs by DMR classes 3-4 and 5-6 and potential damage ratings by habitat in Arizona and New Mexico (from Mathiasen and Blake 1984).

Habitat Type <sup>1</sup>	% Growth Reduction		Potential Damage Rating
	DMR 3-4	DMR 5-6	
PIPU-PIEN/ERSU	31	63	94
ABCO-PSME/ACGL; BERE Ph.	20	65	85
ABCO-PSME; RONE Ph.	25	59	84
ABCO-PSME; BERE Ph.	0	75	75
ABCO-PSME/ERSU	8	65	73
PIPU-PSME; VAAC Ph.	10	55	65
ABCO-PSME/POFE	15	48	63
ABCO-PSME/ ACGL; HODU Ph.	0	60	60
ABLA/ERSU	4	53	57
PIPU-PIEN/SECA	0	45	45
ABLA/VASC-LIBO	0	44	44
ABCO-PSME/QUGA	0	24	24

<sup>1</sup>See Moir and Ludwig 1979 for meaning of habitat type abbreviations.

## CONCLUSIONS

In summary, we can conclude that we know little about the relationship between habitat types and dwarf mistletoe distribution and severity at present. The only pattern that appears to be developing thus far is the relationship of high incidence and severity of dwarf mistletoe infection for two (and possibly three) *Arceuthobium* spp. in habitat types that represent low productivity or xeric sites for their principal hosts (Daubenmire 1961, 1969a, 1969b, Daubenmire and Daubenmire 1968, Schlatterer 1972, Merrill 1983). The majority of information on the incidence and severity of dwarf mistletoe infection by habitat types is primarily qualitative and based on relatively small sample sizes. In addition, only Merrill's (1983) and Fuller and Hofstetler's (1980) reports have been based on random sampling designs. We need to conduct additional studies utilizing random sampling techniques with larger sample sizes taken over a wide geographic range to better understand the relationships between dwarf mistletoe and habitat as stand and fire history, climate, elevation, and topography and dwarf mistletoe habitat types and dwarf mistletoe (Hawksworth 1969, Alexander and Hawksworth 1976, Wicker and Leaphart 1976).

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# The Effect of Dwarf Mistletoe on Forest Community Ecology<sup>1</sup>

Robert O. Tinnin<sup>2</sup>

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**Abstract.**--Dwarf mistletoes cause significant changes in the structure and function of individual host trees. For example, the effects on lodgepole pine include modified rates of physiological process (e.g., decreased rates of respiration for infected trees), different structural characteristics of twigs and needles (e.g., reduced needle and twig mass), and different branch growth patterns associated with witches' broom formation. These effects on individual trees imply several related changes in the communities of which the trees are a part. Indeed, in the stands selected for study, where lodgepole pine was the dominant species, the changes in the individual trees, due to infection by dwarf mistletoe, contribute to visual differences in the structure of the entire community. These structural differences imply functional differences. Quantification of both, and correlation with the level of infection by dwarf mistletoe, is now in progress. This interplay between parasites and community dominants suggests a convenient tool to examine the ecological effects of stress and chronic disturbance on community dynamics.

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## INTRODUCTION

Host-parasite associations are both intimate and complex. They are the result of intricate interrelationships between two species, one of which (the host) provides part or essentially all of the environment for the other (the parasite). Simultaneously the parasite causes changes in its host. The degree of change in the host ranges from slight to extensive, but as it increases, the relationship between the host and its environment also changes. The overall effect is that parasitism can set in motion a chain of events which may have profound effects on communities in which the parasites are active (Holmes 1979).

Parasites are a part of all communities and thereby influence the structure and function of all communities (Price 1980). While this point is known and appreciated, the effect of parasitic plants on plant communities remains to be more completely elucidated. A group of parasites which is superbly suited to the study of parasitic

impact on the dynamics of plant communities are the dwarf mistletoes (*Arceuthobium* spp.). In western North America, dwarf mistletoes are common, easy to find, relatively easy to sample, they have slight to severe effects on their host, and their hosts dominate communities which develop under a wide selection of ecological conditions (Hawksworth and Wiens 1972, Parmeter 1978). That dwarf mistletoes change the structure and function of their host, and thereby of any community of which they are a part, is obvious. The characteristic growth response of most host species, called witches' brooms, illustrates the point (fig. 1). This is but one of the several changes which take place following infection. The point is: what are the quantitative differences between communities with and without dwarf mistletoe?

Parmeter (1978) provided an excellent summary of the dynamics of communities infected with dwarf mistletoe. He noted that these parasites cause a reduction in the growth, wood quality and longevity of their host. The extent of the reduction is dependent on such factors as the number of infections in the tree crown, their position in the crown, the rate of intrapopulation increase, and the extent of witches' broom formation. The extent to which any of these factors affect the host trees depends on many variables (e.g., site quality, host vigor, host resistance, host crown characteristics, stand density, stand age, stand structure and stand

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history). While the multiplicity of interacting factors and variables will make determination of the quantitative effects of parasites on their host community difficult, the answer can nevertheless be obtained.

If an uninfected stand is invaded by dwarf mistletoe, there are several changes in stand structure that can be predicted. One change, having significant economic consequence, is an eventual reduction in stand volume (see Scharpf and Parmeter 1978). Although the effect of infection on tree growth is quite variable, the reduction in stand volume is associated with such things as reduction in bole diameter, height, and/or changes in taper (Baranyay and Safranyik 1970, Hawksworth and Hinds 1964), poor wood quality, mortality, and secondary loss due to insect damage, decay and the increased effect of



Figure 1.--Lodgepole pine infected with lodgepole pine dwarf mistletoe. Notice the large brooms at the base of the crown.

certain abiotic factors (Hawksworth and Wiens 1972). Reductions in the root system of the host have also been reported (Knutson and Toevs 1972). These reductions in growth not only affect individual tree size and thereby stand productivity, but they also affect such ecological processes as competitive interaction. Harper (1977) reports various cases of increased densities of smaller plants in relatively infertile soil and lower densities of larger plants in fertile soil. He suggests that fertile soils, which contribute to increased rates of plant growth, increase "population pressure" leading to fewer plants of greater stature. If dwarf mistletoes decrease host growth, thereby reducing the intensity of competition, heavily infected stands could be expected to be composed of a greater number of smaller trees as compared with uninfected stands.

When an increase in mortality follows infection (Hawksworth and Wiens 1972), one would predict a somewhat greater turnover rate for individuals within the stand. This change would influence host population dynamics which in turn would affect the relative stability of stand composition and/or the rates of stand development in association with successional trends. The potential of increased frequency and intensity of fire in infected stands also contributes to increased mortality (Alexander and Hawksworth 1975, Wicker and Leaphart 1976). Stand conditions, which would otherwise support relatively cool, surface fires, can be changed to conditions which support much more intense fires due to fuel accumulation through the death or senescence of broomed branches or trees. These fires can, of course, rapidly change stand structure and exclude dwarf mistletoe locally. However, these fires can also produce suitable conditions for the reestablishment of young, vigorous, seral stands composed of some host species which otherwise would tend to be eliminated from climax stands. Where dwarf mistletoe attacks these seral species, mistletoes and fire can interact synergistically to maintain stand conditions well suited to the perpetuation of dwarf mistletoe infection.

Changes in the structure of individual trees also leads to the development of unique habitats for some animal species (Tinnin et al. 1982). Animals, or their nests, frequently are found in association with dense brooms.

All of the structural changes noted above are associated with functional changes as well. Among the functional changes which occur are an increase in water loss from the host due to transpirational losses from the aerial shoots of the dwarf mistletoe (Fisher 1975), selective localization of certain minerals in and/or near sites of infection by dwarf mistletoe (McDowell 1963), changes in rates of respiration and photosynthesis (Clark and Bonga 1970), and changes in the occurrence and concentration of growth regulators (Paquet 1979, Schaffer et al. 1983). The significance of these



functional changes has not been completely determined but the fact that they exist points to the reality of significant modification of host metabolism.

#### STUDIES IN PROGRESS

This section summarizes work in progress or work not yet published. Several studies of communities of lodgepole pine (*Pinus contorta*), uninfected and infected with lodgepole pine dwarf mistletoe (*A. americanum*), have been initiated. The communities studied are located on the east flank of the Cascade Mountains in Oregon. These pine forests are ecologically less complex than other forest types so are ideal for a preliminary study of effects of dwarf mistletoe on the dynamics of forest communities. Two different sites have served as the focus of study. Stand density at the sites was 0.3 trees/m<sup>2</sup> at site 1 and 0.2 trees/m<sup>2</sup> at site 2 for the overstory, and 1.2 trees/m<sup>2</sup> and 1.8 trees/m<sup>2</sup> respectively for all lodgepole pine present, regardless of size (Larsen 1981). The mature trees averaged 84 and 88 years old, 25 cm and 26 cm in diameter at breast height and had an average height of 13 m and 14 m respectively for the two sites.

In order to understand the effects of dwarf mistletoe on biomass reallocations within trees, I have begun to examine the difference in growth form between lightly infected and heavily infected trees. Adjacent small trees (<5.2 m tall) were felled a pair at a time and removed to the laboratory. They were dismantled branch by branch and the dry mass (48 hours at 80 C) of the needles and twigs of each was measured. The mean annual mass increment of the heavily infected trees was 87% of the lightly infected trees. Preliminary samples have shown that with severe infection, relatively more mass is found in branch wood than in trunk wood, and the mass of needles to wood tends to be less on heavily infected trees (table 1). The changes in mass are due primarily to the large brooms that form in the lower crown (fig. 1).

Broshot (1982) looked at specific characteristics of needles and twigs and found additional structural differences between healthy and infected trees (table 2). She found a significant decrease in needle number, needle length, needle biomass, twig length and twig mass per annual grown segment for individual twigs from branches which supported dwarf mistletoe. Based on these data and using the procedures of Mellor and Tregunna (1972), she also found a significant decrease in needle surface area.

I am studying the number of needles and twigs in infected and uninfected branches of approximately equal age. The results to date show much higher populations of needles and twigs on systemically infected branches (witches' brooms). The higher population of needles is due to the higher population of twigs rather than more needles per twig. As the number of needles and twigs increases, correspondingly there is an increase in the mass of branch wood and branch needles. However, wood mass tends to increase at a greater rate than photosynthetic tissue (the number of needles). This trend is associated with needles of lesser individual mass (Broshot 1982). Thus, systemic brooms are composed of a greater number and total mass of smaller needles on a more massive and profusely branched limb. These results are similar to those reported for Douglas-fir (Tinnin and Knutson 1980). The implications of these findings are that physiological phenomena, such as moisture loss and CO<sub>2</sub> exchange, are affected.

The data reported above show substantial reallocation of host biomass following infection. These structural changes are associated with several functional changes. Broshot (1982) showed that needles from infected branches contained 2 to 3 times more starch than uninfected needles. The twigs showed a similar but less pronounced response. Similar results are reported for the hosts of other plant parasites as well (Allen 1942, Inman 1962, MacDonald and Strobel 1970, Daly 1976). There are several possible explanations for Broshot's observation. One which is

Table 1.--Relative mass of the aerial portions of lodgepole pine infected by lodgepole pine dwarf mistletoe (sample is too small for valid statistical analysis).

	Mass as Percent of Total		Ratio
	Light <sup>1</sup> Infection	Heavy <sup>2</sup> Infection	(Heavy/Light) <sup>3</sup>
Needles	21	17	.82
Limbs	17	28	1.69
Trunk	62	55	.87
Sample size	4	4	--

<sup>1</sup>No large brooms present and relatively few local infections.

<sup>2</sup>At least one relatively large broom and numerous local infections.

<sup>3</sup>Ratios are based on original mass data, not the percentages reported in this table.

Table 2.--Changes in needle characteristics of lodgepole pine as a result of infection by lodgepole pine dwarf mistletoe (from Broshot 1982).

Mean Needle Characteristics	Site	Branch Infection Type <sup>1</sup>		
		None	Local	Systemic
Number/annual segment	1	85 a	53 b	47 c
	2	58 a	42 b	--
Length (cm)	1	4.1 a	3.4 a	2.1 b
	2	3.5 a	2.9 b	--
Mass/annual segment (g)	1	1.59 a	.68 b	.32 c
	2	.73 a	.39 b	--
Surface Area/annual segment (cm)	1	795.6 a	671.4 b	409.1 b
	2	693.8 a	575.5 b	--
Sample Size	1	4	41	20
	2	13	21	0

<sup>1</sup>Differences shown are significant at the 5% level of confidence.

particularly interesting to me is the possibility that the whole infected branch is a carbon sink on the host tree (Clark and Bonga 1970). Leonard and Hull (1965) reported only limited movement of photosynthate into intact infected branches but they were examining relatively small branches. The sink hypothesis requires further testing.

A related study by Wanner and me (unpublished data) has shown that the metabolic rates of the host are also affected. We found that the rate of photosynthesis of infected trees tends to be somewhat higher than that of uninfected trees. This is consistent with what Clark and Bonga (1970) reported for spruce. However, we found the rate of respiration of infected trees to be significantly lower than that of uninfected trees (table 3). This is not consistent with what Clark and Bonga reported. We also found that the dwarf mistletoe respired significantly faster than either healthy or infected tree tissues, an

observation consistent with McDowell (1963) and Fisher (1975). (There are no data available on the rate of respiration of root tissue in infected trees.) While it is tempting to assume that higher rates of photosynthesis and lower rates of respiration explain the increased levels of starch in the infected host tissues of lodgepole pine, such an assumption is premature until more is known about infected branches as resource sinks.

Dwarf mistletoes are known to affect the reproductive potential of their host. Schaffer et al. (1983) found reductions in cone and seed size on infected trees. Wanner (personal communication) has found significant reductions in cone number as well. He also found a significant decrease in the number of germlings in infected stands although there are noticeably more seedlings and saplings in infected stands. The importance of these changes is now being investigated.

Table 3.--Rates of dark respiration for different tissue samples from lodgepole pine and lodgepole pine dwarf mistletoe.

Tissue Type	n	Rate <sup>1</sup>	p<.05
Needles from uninfected tree	22	286	a
Needles from uninfected branch from infected tree	22	218	b
Needles from infected branch	22	200	b
Aerial shoots	22	438	c

<sup>1</sup> $\mu\text{l O}_2 / \text{g} \cdot \text{hr}$ , standardized to 20°C.



## EFFECTS OF PARASITISM ON COMMUNITIES

The changes in structure and function of host trees noted above provide ample qualitative evidence of changes in the structure and function of the forest community. A great deal is known about infra- and suprapopulations of dwarf mistletoe, and of the effects of dwarf mistletoe on the host population. With regard to fire ecology, there is some information on community dynamics, but with an orientation towards timber management (see Zimmerman and Lavern, these proceedings). What is markedly absent is information on the general ecological effects of dwarf mistletoe on the basic structure and function of the host community as a whole. One is left to ask: what are the changes in community structure and function which occur subsequent to the introduction of dwarf mistletoe?

If one were to characterize important structural features of a forest community, these would include changes in stand density, species composition and diversity, physiognomy and spatial patterns of dispersal. Do dwarf mistletoes affect any of these characteristics? The answer is, of course, yes. As already discussed, changes in mortality, fire frequency and susceptibility to infection all directly affect forest community structure. Similarly, one might characterize productivity and mineral cycling as important functional attributes of a forest community. Again, little is known of the effect of dwarf mistletoe on these processes.

What is known or suspected at the present time is that dwarf mistletoes may contribute to higher stand density, less mass per individual tree, lower canopy height, lower germling densities due to lower seed density per unit area, better seedling survival with higher total seedling density, higher plant and animal species diversity, and greater variation in tree growth form. The effects on such community processes as productivity and nutrient cycling are not apparent to me and clearly need study. These are but a few of the actual changes which eventually will be described. These few points are nonetheless significant as a reminder of how important parasites are in natural ecosystems. Parasites collectively do much to shape plant communities but many of the effects have yet to be evaluated in detail. Dwarf mistletoes are but one genus among the many parasitic genera present and even so have a dramatic effect on the communities of which they are a part. A major reason the effect is so marked is that dwarf mistletoes frequently attack community dominants. Any resulting change in the dominants will be expressed throughout the rest of the community.

In summary, there are many ecological differences between infected and uninfected stands. They exist as differences in both structure and function. No evaluation of communities, without attention to the subtle variable of parasitism, can lead to proper interpretation of community process. The possible applications of such studies would be toward a

better understanding of the effects of mild chronic disturbance and slight increases in stress on forest community structure and function. As such, the potential for study is tremendous and the importance of elucidating these significant forest dynamics would be difficult to overemphasize.

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# Ecological Interrelationships of Dwarf Mistletoe and Fire in Lodgepole Pine Forests<sup>1</sup>

G. Thomas Zimmerman and Richard D. Laven<sup>2</sup>

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**Abstract.**--Dwarf mistletoe (*Arceuthobium americanum* Nutt. ex Engelm.) is the most serious disease agent in lodgepole pine (*Pinus contorta* Dougl.) forests. Fire represents the ecological factor most responsible for the structure and distribution of lodgepole pine forests and the frequency and intensity of dwarf mistletoe infection. In southwestern Colorado lodgepole pine stands, dwarf mistletoe infection intensity and frequency are most closely related to the frequency of fires per year. As the number of fires per year increase, average stand dwarf mistletoe ratings decrease. Other fire history attributes such as the number of years since the last fire and the average length of time, in years, between fires do not appear to strongly influence dwarf mistletoe spread and intensification. Fire exclusion in these forests has not facilitated increased infection by dwarf mistletoe. However, a continuation of this policy, in the absence of alternative mechanisms of regulation, will allow rapid and progressive dwarf mistletoe spread and proliferation.

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## INTRODUCTION

Lodgepole pine is a coniferous forest species which is extremely abundant and widespread throughout the western United States and Canada. The ability of this species to establish and develop under a broad range of environmental conditions and throughout a wide geographic range is greater than nearly every other coniferous species found in North America (Pfister and Daubenmire 1975). This substantially wide ecologic amplitude, in combination with reproductive adaptations that favor establishment and growth following fire, contribute to the wide distribution and importance of these forests.

Fire functions as a dominant force in the development of lodgepole pine forests and may be the single most important factor influencing stand establishment and structure (Wellner 1970, Brown 1975). The variation in intensity occurring during a fire, amount of stored seed available, and the probability of a seedling becoming established influence the post-fire physical development of lodgepole pine stands (Lotan and Jensen 1970, Brown 1975). Differential intensity levels occurring during past fires is responsible for present day stand structure

in the central Rocky Mountains. Extensive even-aged stands are attributable to high intensity fires while the presence of uneven-aged stands confirm the occurrence of low intensity fires (Leiberg 1904, Smithers 1961).

Although fire represents an important and complex force, dwarf mistletoe also constitutes an important ecological factor in lodgepole pine forests. This disease, present in nearly 50 percent of all stands in Colorado, has marked effects on forest growth and structure.

Lodgepole pine dwarf mistletoe is an obligate parasitic seed plant having no fire-enduring or fire-evading mechanisms (Rowe 1983). Its survival then, is contingent upon fire avoidance. But, since the host attacked by this pathogen is fire-dependent, fire-avoidance is difficult.

Fire, therefore, represents an ecological factor capable of either directly or indirectly influencing dwarf mistletoe growth and proliferation. Direct effects result in mortality from tissue oxidation and reduction of the parasite plants to ash, or from destruction of the host tree (Wicker and Leaphart 1976). Indirect effects, such as smoke or temperature influences, may influence parasite reproduction and limit further infection. Such indirect effects can impact the parasite plants much more severely than host trees (Koonce and Roth 1980).

Wildfire is frequently suggested to be the major factor influencing dwarf mistletoe in lodgepole pine forests (Dowding 1929; Gill and Hawksworth 1961;

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Taylor 1969; Baranyay 1970; Heinzelman 1970, 1973, 1978; Kiil 1974; Fellin 1980). However, few, if any, studies have quantitatively established the role of fire occurrence in regulating dwarf mistletoe populations (Hawksworth 1961, 1975; Wright and Heinzelman 1963). In addition, the effects of fire absence on dwarf mistletoe spread and intensification are not well understood (Brown 1975).

This study was undertaken in an attempt to improve our understanding of the reciprocal relations between dwarf mistletoe and fire in lodgepole pine forests. Specific objectives were:

1. To investigate the relationship between dwarf mistletoe frequency and abundance and wildfire history; and
2. To assess the effects of fire suppression on dwarf mistletoe spread and intensification.

#### STUDY AREA

Areas of the Gunnison National Forest in Gunnison County, Colorado served as the study location. Specific study sites were located in the Taylor Park area of the Taylor River Ranger District in southwestern Colorado. Taylor Park, located approximately 61 km northeast of Gunnison, is a high elevation mountain valley bounded on the north and east by the continental divide. Study site elevations range from 2920 to 3200 m and slopes vary from nearly flat to moderately sloping. Fifteen separate sites located throughout Taylor Park were chosen for sampling. Stands were selected on the basis of: the absence of any forest development activity (ie thinning, harvesting, etc.), presence of fire-scarred trees, differences in age classes, and dispersal throughout the study area. Rectangular plots ranging from 150 to 375 m<sup>2</sup> were established in each stand for tree measurements.

#### METHODS

Measurements were taken at each site to provide information regarding dwarf mistletoe infection intensity, time since infection, stand age, and fire history. Dwarf mistletoe infection intensity was evaluated by determining a dwarf mistletoe rating for each tree tallied in each plot and then computing an average stand dwarf mistletoe rating (DMR). Dwarf mistletoe ratings for each tree were determined by using the 6-class rating system (Hawksworth 1977). Average stand rating (DMR) was calculated by totaling individual tree ratings and dividing by the total number of trees.

Time since infection by dwarf mistletoe (TSI) was determined by a method similar to one described by Scharpf and Parmeter (1966). Cross sections were removed from infected stems and examined with a microscope to facilitate a count of the annual growth rings from the bark to the ring nearest the innermost portion of the longest parasite sinker. Stem infections suitable for determining time since infection were only found in three plots. In the remaining plots, time since infection was estimated

indirectly from average stand dwarf mistletoe ratings. Using data presented by Hawksworth and Hinds (1964), the following relationships between DMR and time since infection were developed:

If average stand DMR greater than 0 but less than .6, then time since infection = average stand DMR/.06

If DMR greater than .6 but less than 2.2, then time since infection = ((average stand DMR - 2.2)/.08) + 10

If DMR greater than 2.2 but less than 4.0, then time since infection = ((average stand DMR - 2.2)/.09) + 30

If DMR greater than 4.0 but less than 4.6, then time since infection = ((average stand DMR - 4.0)/.06) + 50

If DMR greater than 4.6, then time since infection = ((average stand DMR - 4.6)/.03) + 60

Tree age was assessed by collecting two increment cores at ground level from a random sample of 25 percent of all live trees. Age was estimated by examining the cores under a microscope and counting annual rings.

Fire history was determined from dating of fire scar samples from live trees. Complete cross sections were removed from at least two fire-scarred trees at each sampling site. Dates of fires were determined for each of these trees by counting, with the aid of a microscope, the number of annual rings from the sampling year (1983) to the most recent scar and between previous scars.

For each sampling area the mean fire return interval was calculated by deriving the arithmetic average of the number of years between each two successive fires, except when only one fire event was documented. In this instance, the fire return interval was taken as the time from that fire to the present and will continue to increase until the next fire occurs. Time since the last fire was estimated by determining the number of years from the sampling year to the most recent fire event. Fire frequency was computed by dividing the number of fires recorded for each site by the age in years of the oldest living fire-scarred tree sampled in 1983.

Stage II inventory data on file at the Taylor River Ranger District were also reviewed to gain additional dwarf mistletoe rating and stand age information. Average stand age and DMR data from 122 additional lodgepole pine stands were reviewed and used for comparison with age and DMR data collected during this study.

#### RESULTS AND DISCUSSION

##### Dwarf Mistletoe Infection Intensity

Dwarf mistletoe infection was present, to some degree, in 100 percent of the stands sampled in this study. When compared by individual DMR class inter-



vals, the greatest percentage of stand were infected at the highest level (DMR = 5.1-6.0), followed by the immediately lower two class intervals (DMR = 4.1-5.0 and 3.1-4.0). Distribution of lodgepole pine stands by DMR class intervals in Taylor Park was:

DMR class interval	Percentage of stands infected
0	0
0.1 - 1.0	13.3
1.1 - 2.0	6.7
2.1 - 3.0	6.7
3.1 - 4.0	20.0
4.1 - 5.0	20.0
5.1 - 6.0	33.3

Over 70 percent of these lodgepole pine stands contained dwarf mistletoe infections with intensities greater than 3.1.

#### Time Since Infection

Because the time since infection was computed on the basis of the average stand dwarf mistletoe rating, it closely followed the trend observed in infection intensity levels. Specifically, as DMR increased so did the time since infection. Consequently, over 70 percent of all sampled stands had been infected for 40 years or longer.

For those stands where stem infections were used to age infections, time since infection was also computed by the same method as for other stands. For all three stands, the age of the stem infection was very close to the estimated time since infection. In one stand, the two methods produced an identical time since infection.

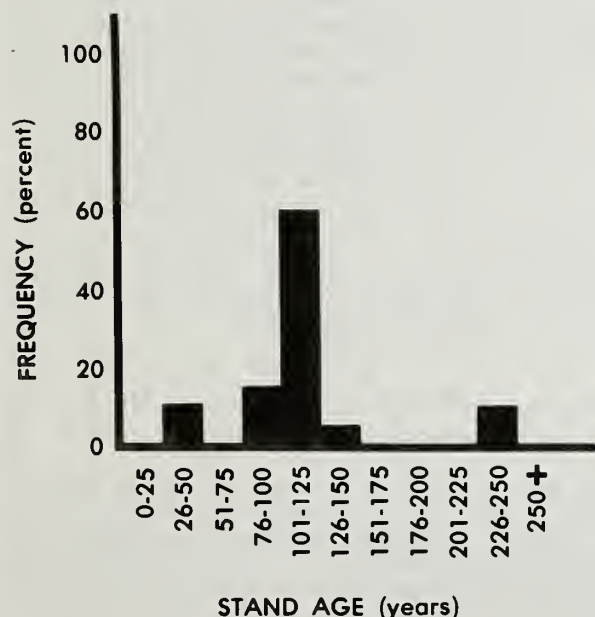


Figure 1.--Frequency histogram of lodgepole pine stand age in Taylor Park, Colorado.

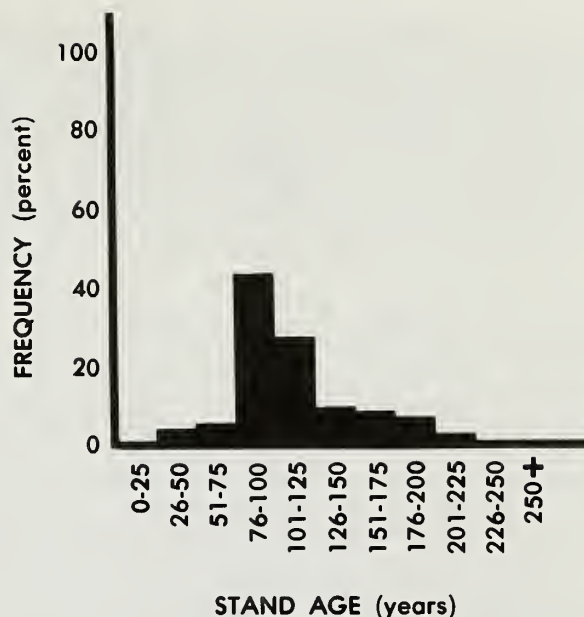


Figure 2.--Frequency histogram of lodgepole pine stand age in Taylor Park, Colorado (source = USFS inventory data).

#### Stand Age

Analysis of age class distribution of lodgepole pine stands in the Taylor Park area revealed that stands were most frequently between 101 and 125 years old (fig. 1). Sixty percent of all measured stands occurred within this age range. Stands representing young and mature age classes were noticeably lacking (fig. 1).

Analysis of the age class distribution of the additional stands surveyed in the Stage II forest inventory revealed similar trends. The greatest percentage of stands were found in age classes of 76-100 and 101-125 years (fig. 2). These age ranges consisted of 43 and 26 percent of all stands respectively. Again, young age stands were lacking; only eight percent of all stands were between 1 and 75 years in age. Frequency of stands older than 125 years showed a continual decline with increasing age (fig. 2).

#### Fire History

The most active period of fire occurrence in the Taylor Park area as indicated from fire scar analyses was between 1858 and 1882 (101 to 125 years before the measurement year, 1983) (fig. 3). Numbers of fires occurring before the peak were much lower. A decline in fire occurrence, as indicated by scarred trees, occurred during the last 100 years.

Considerable variation between the sampled stands was observed in the time since the last fire, mean fire return interval, number of fires during the life of the stand, and fire frequency (table 1). Time since the last fire varied from as low as seven to as high as 132 years. Observed mean fire return intervals were as low as four years and as high as

Table 1.--Fire history characteristics and stand age of sampled stands in Taylor Park, Colorado.

Stand #	Ave. Age (years)	Time since last fire (years)	Mean fire return interval (years)	Number of fires	Fire frequency (fires/year)
09	111	47	41	3	.013
11	47	7	17	7	.056
12	122	102	10	6	.023
13	33	51	10	4	.047
14	115	104	4	4	.032
17	107	22	25	5	.022
18	109	50	38	3	.013
19	93	103	27	4	.017
38	124	24	31	8	.029
139	114	132	133+ <sup>1</sup>	1	.003
239	227	132	133+ <sup>1</sup>	1	.003
140	122	121	70	3	.010
240	236	121	70	3	.010
141	83	92	8	6	.028
241	103	92	8	6	.028

<sup>1</sup>This stand has only one fire record, thus, the fire return interval has been taken as the time from that fire to the present and will continue to increase until the next fire occurs.

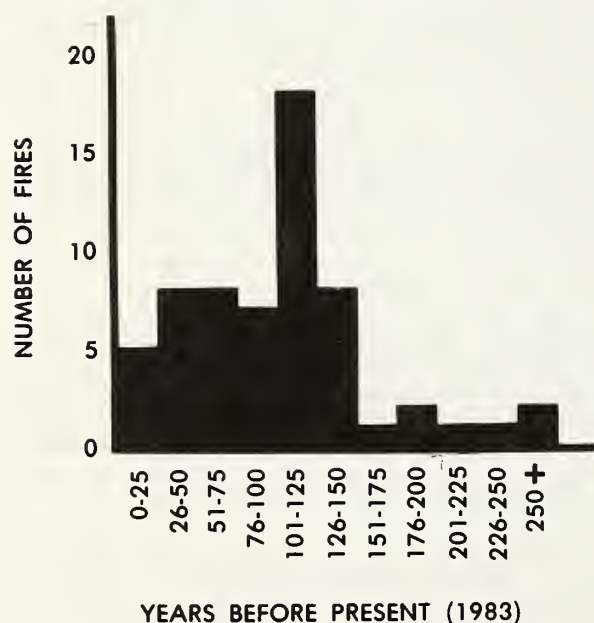


Figure 3.--Number of fires during 25 year periods in Taylor Park, Colorado as determined from scarred lodgepole pine trees.

at least 133 years. Lodgepole pine forests are considered to have a variable fire regime consisting of frequent, low-intensity surface fires and infrequent, high-intensity stand destroying fires (Kilgore 1981). The fire return intervals reported here approximate such a fire regime although some return intervals are lower than reported by other authors (Arno 1980). The number of fires that occurred during the life of each stand ranged from one to eight. Over half the stands had been burned at least four times during their life span (table 1). Fire frequency, expressed in fires per year, was extremely low, although the highest was nearly 20 times greater than the lowest frequency (table 1).

#### Relationships Between Dwarf Mistletoe Infection and Fire History

In figure 4, DMR is plotted against the mean fire return interval. Superimposed on this figure is a line which represents the rate of dwarf mistletoe intensification over time. This line illustrates the relationship between DMR and time since infection presented by Hawksworth and Hinds (1964). This relationship is plotted on this figure because it was expected that a plot of DMR versus the mean fire



return interval would have data points clustered about this line. However, this was not the case; data points are scattered over the graph. Interpretation of this figure in terms of the time since infection and time since the last fire does, however, provide a possible explanation for some of the variation. Those stands where dwarf mistletoe infection has been present longer than the time since the last fire represent situations where the most recent fire did not eradicate the parasite from the stand. Consequently, DMR escalation during the subsequent fire-free period appears very rapid. In those stands infected since the last fire, the disease has probably spread from outside the stand and these points are scattered around the projected rate of DMR intensification over time. Two stands plotted in the upper left-hand corner of the figure, however, are not accounted for by either of these explanations.

The mean fire return interval merely reflects an arithmetic average. Some very long return intervals in combination with very short intervals could yield a misleading average. So, mean fire return intervals appear limited in their usefulness for relating dwarf mistletoe infection history to fire history. This fact was borne out during linear regression analyses of these data which produced an equation with  $R^2 = .13$ . Length of time from the last fire in combination with the most recent fire return interval may be more informative when attempting to relate dwarf mistletoe infection history to fire history.

The intensity with which a fire burns is directly responsible for the type of post-fire infection source (ie, even, uneven, or peripheral (Baranyay 1970)), and can both encourage and discourage dwarf mistletoe spread and intensification (Hawsworth 1969). High intensity fires that consume all or nearly all of a stand, causing simultaneous death of all infected trees and parasite plants, tend to discourage dwarf mistletoe development and spread. Following this type of fire a sanitized stand results in which the reinvading tree regeneration develops at a faster rate than the parasite (Jones 1974). On the other hand, fires that burn sporadically

permit the survival of infected trees (even or uneven infections) which spread the pathogen to regeneration and encourage dwarf mistletoe intensification (Kimney and Mielke 1959, Graham 1960, Hawsworth 1969, Muir 1970). If fires kill all trees in a single stand but leave surrounding infected stands undisturbed, then peripheral infections occur. In these instances, the pathogen would be expected to increase at a rate somewhere between the two previously mentioned extremes.

The role of fire in encouraging or discouraging dwarf mistletoe is clearly illustrated when DMR is plotted against time since the last fire, with the relationship between parasite intensification and time since infection again plotted as a line (fig. 5). Those points above the line represent stands where fire has encouraged dwarf mistletoe intensification. Incomplete consumption of infected trees permitted reinfection of regeneration and rapid escalation of the DMR. Those points below and to the right of the intensification line represent stands where fire has discouraged dwarf mistletoe. In these stands, high intensity fires have probably killed all infected trees and effectively eradicated the disease. Spread from surrounding infection centers has been slow. Immediately adjacent to the intensification line are those stands where fire was responsible for removal of dwarf mistletoe, and reinfection progressed steadily from peripheral infection sources.

If fire occurrence is important in dwarf mistletoe regulation, then increasing fire frequency should result in decreasing DMR levels. The relationship for the Taylor Park stands is illustrated in Figure 6. Dwarf mistletoe rating clearly decreases as fire frequency increases. Linear regression analysis yielded the following equation for predicting DMR from fire frequency:

$$DMR = 5.485 - 79.574 * (\text{FIRE FREQUENCY})$$

This equation accounts for 47 percent of the variation among the sampled stands and is plotted in figure 6.

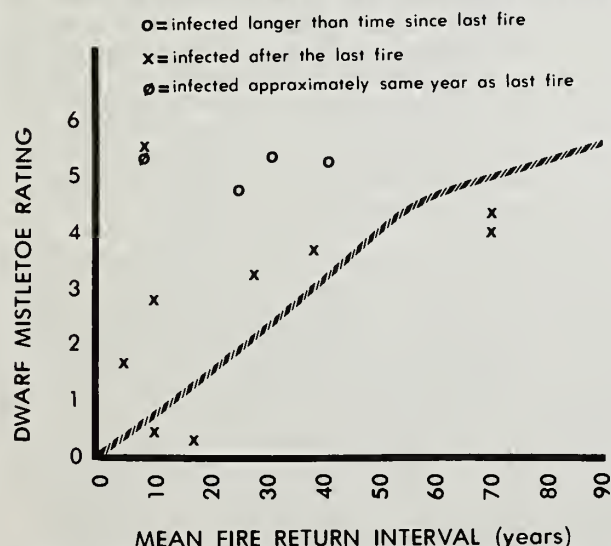


Figure 4.--Relationship between average stand dwarf mistletoe rating and mean fire return interval.

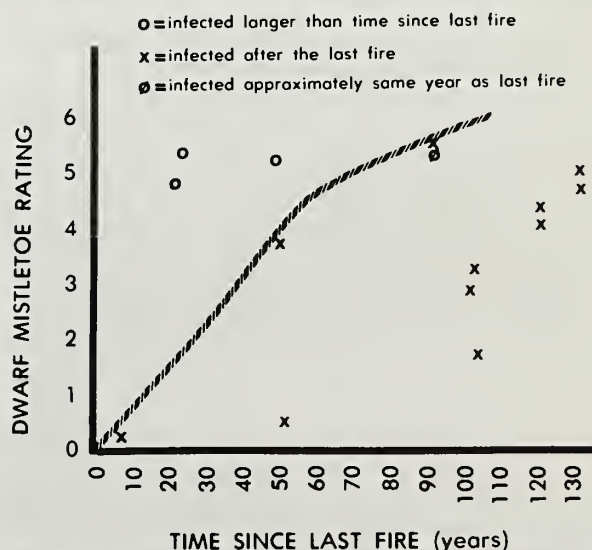


Figure 5.--Relationship between average stand dwarf mistletoe rating and time since the last fire.

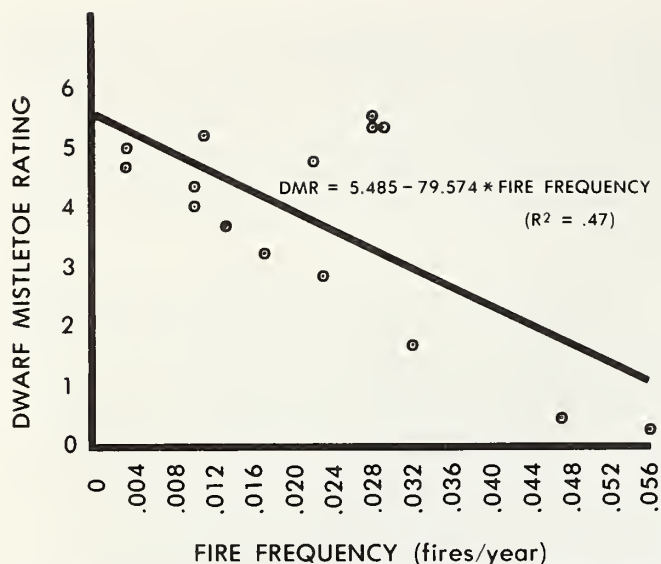


Figure 6.--Relationship between average stand dwarf mistletoe rating and fire frequency.

#### Effects of Fire Exclusion on Dwarf Mistletoe Spread and Intensification

Fire exclusion effects on dwarf mistletoe spread and intensification are not well understood (Brown 1975). A policy of wildfire suppression is thought to have removed the only natural control and facilitated an increase in dwarf mistletoe in lodgepole pine forests during the past few decades (Kimmey 1957; Baranyay 1970, 1975; Heinzelman 1970; Smith and Baranyay 1970; Loope 1971; Hawksworth 1975). However, in those stands where shade tolerant species succeed lodgepole pine, fire exclusion could indirectly reduce dwarf mistletoe infection by reducing the numbers of lodgepole pine and increasing the numbers of shade tolerant individuals (Bloomberg 1950, Brown 1975). Since most of these shade tolerant species are immune to lodgepole pine dwarf mistletoe (Hawksworth 1975), a decrease in infection centers will result.

Within lodgepole pine forests fire behavior cycles are believed to follow definite trends (Muraro 1971, Brown 1975, Van Wagner 1983). Lodgepole pine fire intensity potential which refers to fire growth and heat release potential, is illustrated over time in figure 7 (Brown 1975). The most frequent occurrence believed to occur in these stands is shown by curve A. Young stands, especially dense ones, possess the greatest fire potential while moderately dense to open advanced immature and mature stands have the least fire potential (Muraro 1971; Brown 1975; Van Wagner 1977, 1983). Potential fire intensity increases rapidly to a maximum during the initial 20-30 years of stand development, although intense fires are possible in forests as young as 10 years (Van Wagner 1983). Potential fire intensity then decreases until stand deterioration where it again increases (Muraro 1971, Brown 1975, Van Wagner 1983). Curve C represents situations where surface fuel quantities and fire potential remain relatively low throughout the life of the stand until decadence (Brown 1975). Potential fluctuation between these

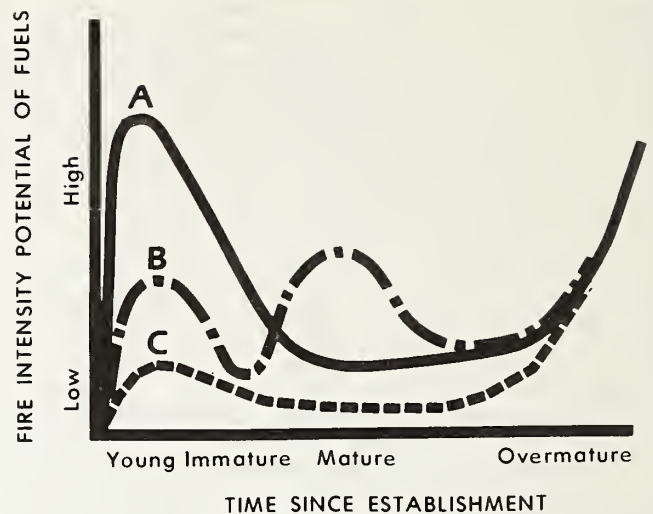


Figure 7.--Fire intensity potential cycle in lodgepole pine forests (Brown 1975).

two curves during younger ages and prior to increased fire intensity at later ages is illustrated by curve B (Brown 1975).

Thus, if a regular trend in fire behavior potential exists in lodgepole pine stands, it is likely that a similar trend in the number of fires also exists. Maximum numbers of fires probably occur during periods when a large percentage of stands have reached the most hazardous stages. This trend is apparent for stands in the Taylor Park area (fig. 4). Fire occurrence, derived from fire-scarred trees, increased from 1723 to a peak between 1858 and 1892 and then decreased. If dwarf mistletoe presence is related to fire occurrence, then dwarf mistletoe frequency should be at a minimum shortly after the peak of fire occurrence. Number of fires or number of stands burned and percent of stands infected by dwarf mistletoe for Taylor Park lodgepole pine stands are plotted for 10 year periods from 1713 to 1983 in figure 8. The pattern of fire occurrence subdivided into 10 year intervals appears to further substantiate a fire cycle for this area. In addition, in 1983 100 percent of the sampled stands were infected by dwarf mistletoe while approximately 95 years ago, none of these stands were infected. This minimum in the frequency of dwarf mistletoe infection lags slightly behind the peak years of fire occurrence.

If lodgepole pine stands in the Taylor Park area are subject to a fire occurrence cycle, then, during the twentieth century, fire occurrence has followed a decline in response to declining stand fire behavior potential. Consequently, dwarf mistletoe infection intensity and frequency have followed a cyclic increase which will persist until the next peak in fire occurrence.

Although the effects of fire suppression have not been as serious in these lodgepole pine stands as previously believed, continued fire exclusion could have serious ramifications. These lodgepole pine stands now have a very high percentage of dwarf



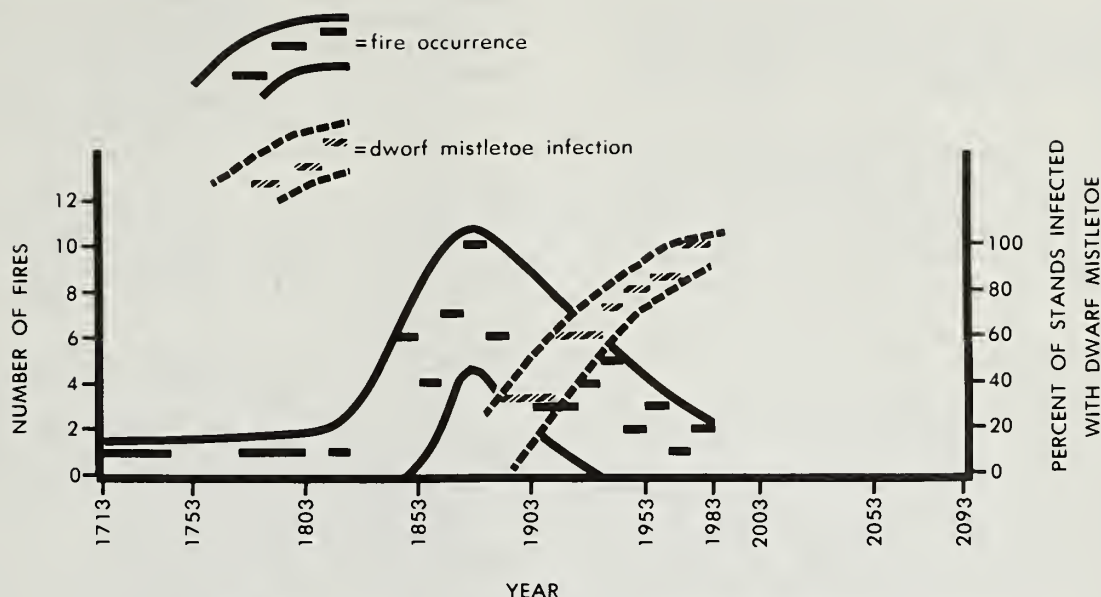


Figure 8.--Fire and dwarf mistletoe dynamics in lodgepole pine stands of Taylor, Colorado.

mistletoe infection. A continuation of the fire suppression policy in the absence of alternative dwarf mistletoe control or eradication methods will permit continued parasite spread and proliferation and drastically reduce stand productivity. In addition, continued fire suppression could decrease the number of stands of different ages and the large number of young stands usually associated with fire-dependent forests. A mosaic of few rather than many age classes will result where a large percentage of stands will reach a stage of high potential fire intensity at the same time which could drastically affect forest protection problems.

#### SUMMARY

The relationship between fire and dwarf mistletoe is difficult to quantitatively document. But in lodgepole pine stands in the Taylor Park area of southwestern Colorado certain trends are apparent. Fire frequency appears to be more useful than either mean fire return interval or time since the last fire for relating dwarf mistletoe infection intensity to fire history. Average stand dwarf mistletoe ratings of lodgepole pine stands in Taylor Park are inversely proportional to the fire frequency recorded in fires per year. The usefulness of this relationship should, however, be interpreted with caution. This study indicates lodgepole pine stands in Taylor Park, Colorado are subject to a variable fire regime: longer interval stand destroying fires superimposed on more frequent fires of lesser intensities. Areas outside the limits of this study could be subject to far different fire regimes where the relationship between dwarf mistletoe infection and fire history is unlike that reported here.

Wildfire suppression over the past few decades in lodgepole pine stands in Taylor Park, Colorado has not resulted in increased dwarf mistletoe

infection intensity and frequency. The increase that has occurred is instead a predictable result of the dwarf mistletoe and fire dynamics of this area. But, continued fire suppression in these lodgepole pine stands, in the absence of alternative dwarf mistletoe controls, will permit dwarf mistletoe to continue to spread and intensify. Ultimately, severe losses in stand productivity will occur.

Because of its parasitic nature, dwarf mistletoe is adversely affected by any factor that harms host trees. Lodgepole pine stands in the Taylor Park area are strongly influenced by wildfire occurrence. Fire represents one of the foremost natural agents of dwarf mistletoe regulation and influences parasite presence and abundance through affects on both the parasite plants and lodgepole pine hosts.

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